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(54) Title: DERIVATIVES OF EPOTHILONE B AND D AND SYNTHESIS THEREOF

(57) Abstract: The present invention provides the following new compounds: (*R*)-C14-methyl-epothilone B, (*S*)-C14-methyl-epothilone B, (*S*)-C14-methyl-epothilone D, and (*R*)-C14-methyl-epothilone D and methods for synthesizing these compounds.

DERIVATIVES OF EPOTHILONE B AND D AND SYNTHESIS THEREOF

GOVERNMENT INTEREST STATEMENT

This invention is made with government support under Grant ID No. CA84599 awarded by the National Cancer Institute/NIH. The government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to derivatives of epothilone B and D.

Description of the Prior Art

Cancer still represents a major unmet medical need. Initial treatment of the disease is often surgery, radiation treatment or the combination, but recurrent (metastatic) disease is common. Chemotherapeutic treatments for most cancers are generally not curative, but only delay disease progression. Commonly, tumors and their metastases become refractory to chemotherapy, in an event known as development of multidrug resistance. In many cases, tumors are inherently resistant to some classes of chemotherapeutic agents.

Among cytotoxic agents for the treatment of tumors, TAXOL® (paclitaxel), a microtubule stabilizing agent, has become a very important compound with a remarkable economic success. However, TAXOL® has a number of disadvantages. Especially its extremely low solubility in water represents a severe problem. It has become necessary to administer TAXOL® in a formulation with Cremophor EL® (polyoxyethylated castor oil; BASF, Ludwigshafen, Germany) which has severe side

effects, causing inter alia allergic reactions that in one case even were reported to have led to the death of a patient. More severely, certain tumor types are known to be refractory to treatment with TAXOL® even when the drug is administered as front-line therapy, or the tumors develop resistance to TAXOL® after multiple cycles of exposure.

Although the taxane class of antimicrotubule anti-cancer agents has been hailed as the perhaps most important addition to the chemotherapeutic armamentarium against cancer over the past several decades and despite the commercial success of TAXOL®, there remain limitations to TAXOL®'s efficacy. TAXOL® treatment is associated with a number of significant side effects and some major classes of solid tumors, namely colon and prostate, are poorly responsive to this compound. For example, the effectiveness of TAXOL® can be severely limited by acquired drug resistance mechanisms occurring via various mechanisms, such as overexpression of phosphoglycoproteins that function as drug efflux pumps.

Therefore, there exists an urgent need to find compounds and appropriate dosing regimens with these compounds to expand the armamentarium of cancer treatment, especially in the majority of cases where treatment with taxanes and other anticancer compounds is not associated with long term survival.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide new compounds that may be used effectively in cancer chemotherapy.

It is a further object of the present invention to provide compounds that may be used to treat TAXOL®-resistant tumors.

It is yet a further object of the present invention to provide new derivatives of epothilone B.

It is yet a further object of the present invention to provide new derivatives of epothilone D.

According to a first broad aspect of the present invention, there is provided compound comprising *(R)*-C14-methyl-epothilone B.

According to second broad aspect of the present invention, there is provided a compound comprising (*S*)-C14-methyl-epothilone B.

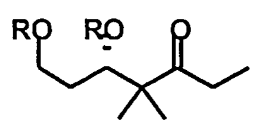
According to third broad aspect of the present invention, there is provided a compound
comprising (*S*)-C14-methyl-epothilone D.

According to fourth broad aspect of the present invention, there is provided a compound comprising (*R*)-C14-methyl-epothilone D

According to a fifth broad aspect of the present invention, there is provided a method for synthesizing (*R*)-C14-methyl-epothilone B comprising the steps of: providing (*S*)-C14-methyl-epothilone D; and (b) incorporating an epoxide group at the C12-C14 position of (*S*)-C14-methyl-epothilone D to form (*R*)-C14-methyl-epothilone B.

According to a sixth broad aspect of the invention, there is provided a method for synthesizing (*S*)-C14-methyl-epothilone D comprising the following steps:

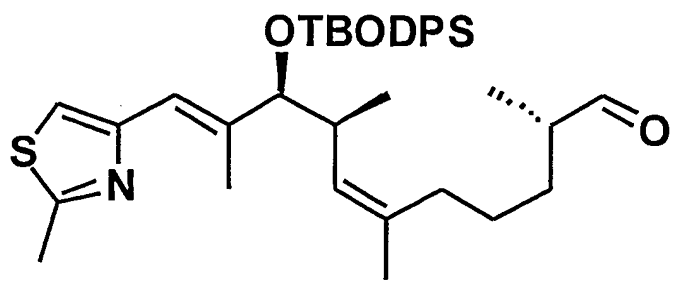
(a) providing an ethyl ketone 10 having the following formula:



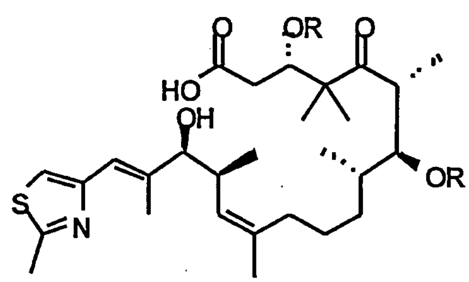
;

(b) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

(c) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



to thereby form a *syn.*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl; (d) exposing said *syn.*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether; (e) de-protecting said *syn.*, *anti*-aldol adduct to form a primary alcohol; (f) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid; (g) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:



;

(h) macrolactonizing compound 11 to form a 16-membered lactone; and (i) treating said 16-membered lactone with TFA to form (*S*)-C14-methyl-epothilone D.

Other objects and features of the present invention will be apparent from the following detailed description of the preferred embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described in conjunction with the accompanying drawings, in which:

FIG. 1 illustrates two conformations of epothilone B and two derivatives of epothilone B;

FIG. 2 illustrates a first scheme of a method of the present invention for forming first intermediates useful in synthesizing the epothilone B and D derivatives of the present invention;

FIG. 3 illustrates a second scheme of a method of the present invention, using the first intermediates of Figure 2, for forming second intermediates useful in synthesizing the epothilone B and D derivatives of the present invention; and

FIG. 4 illustrates a third scheme of a method of the present invention, using the second intermediates of Figure 3 to form the epothilone B and D derivatives of the present invention; and

FIG. 5 illustrates a method of the present invention for forming an epothilone B derivative of the present invention from an epothilone D derivative of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

It is advantageous to define several terms before describing the invention. It should be appreciated that the following definitions are used throughout this application.

Definitions and Abbreviations

For the purposes of the present invention, the term "scheme" refers to one or more sub-steps of a method for synthesizing an epothilone derivative of the present invention.

The following abbreviations appear in Schemes 1, 2 and 3 of FIGS. 2, 3 and 4, respectively: Z-crotyl-(+)-Ipc_2B (Z-3-methylpropenyl-diisopinocampheyl borane) , described in Brown, H. C. and Bhat, K. S. "Chiral Synthesis via Organoboranes. 7. Diastereoselective and Enantioselective Synthesis of *Erythro-* and *Threo*–β–methylhomoallyl Alcohols via Enantiomeric (Z)- and (E)-Crotylboranes" *J. Am. Chem. Soc.* 1986, *108*, 5919-5923, the entire contents and disclosure of which is hereby incorporated by reference), NMO (4-methylmorpholine N-oxide) LDA (lithium diamine), TBODPSCl (*t*-butoxydiphenylsilyl chloride), TBSOTf (t-butyl-dimethylsilyltriflate) TBS (t-butyl-dimethylsilyl), CSA (camphorsulfonic acid), DMP (Dess-Martin periodinane, described in Dess D. B.; Martin J. C. "Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones" *J. Org. Chem.* 1983, *48*, 4155-4156, the entire contents and disclosure of which is hereby incorporated by reference), TBAF (tetrabutylammonium fluoride, TCBCl (2,4,5-tichlorobenzoyl chloride), DIEA (N,N-diisopropylethylamine), DMAP (4-(dimethylamino)pyridine), TFA (trifluoroacetic acid), and mCPBA (m-Chloroperoxy benzoic acid).

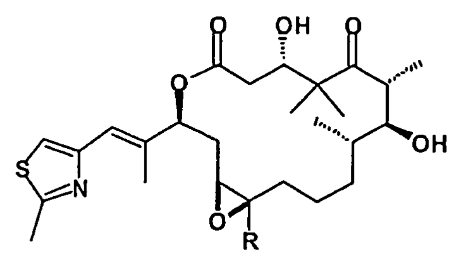
Description

The success of the anticancer drug Taxol®, marketed by Bristol-Myers Squibb, appears to be related to its unique mode of action, tubulin polymerization and microtubule stabilization. However, Taxol®'s low water solubility and inactivity against multi-drug resistant tumors limits Taxol®'s usefulness. Compounds with biological activity related to Taxol®'s activity included the epothilones, but the known epothilones and epothilone derivatives are not satisfactory for all purposes. Several years ago, a study by Taylor *et al.* on the conformational properties of the epothilones which utilized

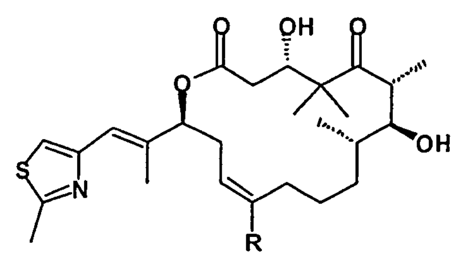
a combination of computational methods and high field NMR experiments, see Taylor, R. E. and Zajicek, J. "The Conformational Properties of Epothilone" *J. Org. Chem.* 1999, *64*, 7224. The Taylor *et al.* study concentrated on the critical C1-C8 polypropionate region and concluded that in solution the epothilones preferred to exist in at least two conformational families controlled by the *syn*-pentane interactions. In addition, the Taylor *et al.* study showed that the major contributor was indeed related to the conformation observed in the solid state, see also Hoefle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; and Reichenbach, H. "Epothilone A and B-Novel 16-membered Macrolides with Cytotoxic Activity: Isolation, Crystal Structure, and Conformation in Solution" *Angew. Chemie* 1996, *35*, 1567-1569. The conversion of an allylic alcohol to an allylic chloride useful in synthesizing epothilone derivatives is described in Taylor, R. E.; and Chen, Y. "The Total Synthesis of Epothilones B and D" *Organic Lett.* 2001, *3*, 2221, the entire contents and disclosure of which is hereby incorporated by reference.

The present invention provides epothilone derivatives and method of making epothilone derivatives based on the stabilization of conformational families through simple substitution. Several of the preferred epothilone derivatives of the present invention have significant biological activity and together provide new insights into the biological active conformation of the epothilone class of natural products.

The chemical formulas for epothilones A, B, C and D are shown below:



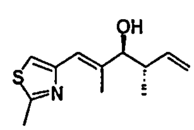
1a R = H epothilone A
2a R = Me epothilone B



1b R = H epothilone C
2b R = Me epothilone D

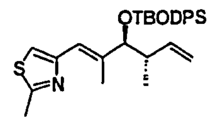
With respect to the C10-C15 epoxide region of epothilone B and epothilone B derivatives, a high degree of flexibility leading to two conformational families shown as Conformer A and B, in which R = H for epothilone B and R = Me for the epothilone B derivative 3 ((*R*)-C14-methyl epothilone B) and epothilone B derivative 4 ((*S*)-C14-methyl epothilone B) of FIG. 1. The epothilone B derivatives 3 and 4 are intriguing because epothilone B derivatives 3 and 4 represent an acetate to propionate modification in the biogenetic pathway. Therefore, advances in genetic engineering of polyketide synthases, see Khosla, C. "Natural Product Biosynthesis: A New Interface between Enzymology and Medicine" J. Org. Chem. 2000, *65*, 8127-8133, may provide alternative methods, in addition to the chemical synthesis methods described below, for producing epothilone B derivative 3 through manipulation of the epothilone gene cluster, see Julien, B.; Shah, S.; Ziermann, R.; Goldman, R.; Katz, L.; and Khosla, C. "Isolation and Characterization of the Epothilone Biosynthetic Gene Cluster from *Soranium cellulosum*" *Gene* 2000, *249*, 153-160. b) Tang, L.; Shah, S.; Chung, L.; Carney, J.; Katz, L.; Khosla, C.; and Julien, B. *Science* 2000, *287*, 640-642. c) Molnar, I.; Schupp, T.; Ono, M.; Zirkle, R.; Milnamow, M.; Nowak-Thompson, B.; Engel, N.; Toupet, C.; Stratmann, A.; Cyr, D. D.; Gorlach, J.; Mayo, J. M.; Hu, A.; Goff, S.; Schmid, J.; and Ligon, J. M. "The Biosynthetic Gene Cluster for the Microtubule-Stabilizing Agents Epothilones A and B from Sorangium cellulosum So ce90" *Chem. Biol.* 2000, *7*, 97-109, or through semi-synthesis/biosynthetic techniques used on *Sorangium cellulosum*, the organism from which epothilones are generally produced.

In a preferred method of the present invention, (*S*)-C14-methyl-epothilone D is synthesized using thiazole aldehyde 5 of Scheme 1 of FIG. 2. A brown asymmetric crotylboration using Z-crotyl-(+)-Ipc_2B to form compound 5a having the following formula:



(5a)

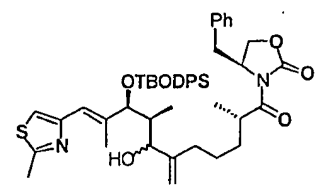
Compound 5a is treated with TBODPSCl to protect the secondary hydroxyl of Compound 5a as t-butoxydiphenylsilyl ether and form compound 5b having the following formula:



(5b)

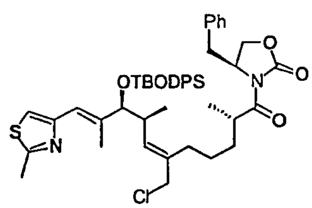
An oxidative cleavage of the terminal alkene using OsO_4, NMO followed by $NaIO_4$ may be performed on compound 5b to form aldehyde 6 of Scheme 1.

Next, a vinyl aldehyde 8, shown in Scheme 2 of FIG. 3, is intermolecularly coupled with aldehyde 6 using Ni/Cr coupling to provide an intermediate allylic alcohol 8a having the following formula as a mixture of diastereomers (1:1). :



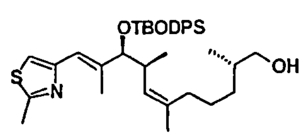
(8a)

Exposure of this mixture to thionyl chloride in ether-pentane provides a primary allylic chloride 8b having the following formula:



(8b)

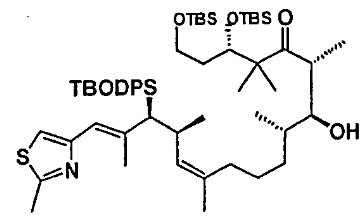
Reacting the primary allylic chloride 8b with $LiEt_3BH$ form a primary alcohol 8c having the following formula:



(8c)

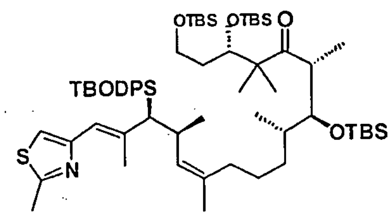
Primary alcohol 8c is then oxidized with Dess-Martin periodane to provide aldehyde 9 of Scheme 2.

Next, ethyl ketone 10 shown in Scheme 3 of FIG. 4 is reacted with LDA and aldehyde 9 to form a *syn, anti*-aldol adduct 11a having the following formula:



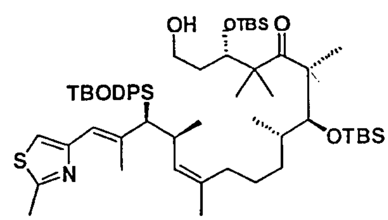
(11a)

The C7 hydroxyl of *syn, anti*-aldol adduct 11a is protected as a TBS ether by reacting *syn, anti*-aldol adduct 11a with TBSOTf to form an etherized adduct 11b having the following formula:



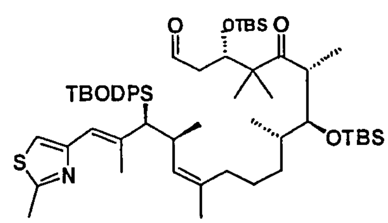
(11b)

Etherized adduct 11b is exposed to acidic methanol solution to liberate a primary alcohol 11c having the following formula:



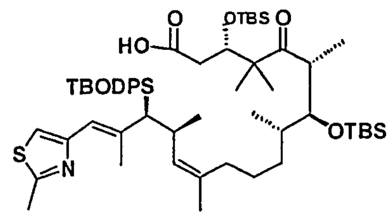
(11c)

Primary alcohol 11c is the treated with DMP to provide aldehyde 11d having the following formula:



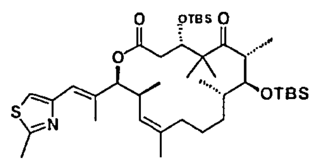
(11d)

Aldehyde 11d is then oxidized to a carboxylic acid, oxidized adduct 11e having the following formula:



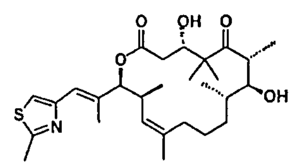
(11e)

Oxidized adduct 11e is then treated with TBAF to remove the C15 TBSDPS ether to form alcohol 11 of Scheme 3 of FIG. 4. A macrolactonization is then carried out on alcohol 11 using TCBCl, DIEA and DMAP to form a 16-membered lactone 12a having the following formula:



(12a)

A deprotection of the C3 and C7 silyl ethers of lactone 12a is then carried out using TFA to form (*S*)-C14-methyl epothilone D 12b having the following formula:



(12b)

In a preferred method of the present invention, (*R*)-C14-methyl-epothilone B is synthesized by exposing (*S*)-C14-methyl-epothilone D to mCPBA.

A method similar to the method described above for synthesizing (*S*)-C14-methyl-epothilone D from thiazole aldehyde 5 may be used to synthesize (*R*)-C14-methyl-epothilone D by carrying out the crotylboration of thiazole aldehyde 5 with E-crotyl-(+)-Ipc_2B instead of Z-crotyl-(+)-Ipc_2B to form aldehyde 7 instead of aldehyde 6.

The remaining synthesis steps to form (*R*)-C14-methyl-epothilone D from aldehyde 7 are similar to the synthesis steps used to form (*S*)-C14-methyl-epothilone D from aldehyde 6.

In a preferred method of the present invention, (*S*)-C14-methyl-epothilone B may be synthesized by exposing (R)-C14-methyl-epothilone D to mCPBA.

EXAMPLES

Example 1

(*R*)-C14-methyl epothilone B (epothilone B derivative 3) was synthesized using a method shown in Schemes 1, 2 and 3 of FIGS. 2, 3 and 4, respectively.

Readily available thiazole aldehyde 5, a common intermediate in several synthetic routes to these epothilone, is the point divergence for the synthesis of epothilone B derivatives 3 and 4 as shown in Scheme 1. Brown asymmetric crotylboration[9] efficiently controlled the enantioselectivity as well as the diastereoselectivity of the C14, 15 stereogenic centers. Protection of the secondary hydroxyl as a t-butoxydiphenylsilyl ether followed by oxidative cleavage of the terminal alkene provided aldehydes 6 and 7.

The conversion of aldehydes 6 and 7 to epothilone B derivatives 3 and 4, respectively, proceeded though identical synthetic sequences. Only the synthesis of epothilone B derivative 3 from aldehyde 6 is described below and shown in Schemes 2 and 3, of FIGS. 3 and 4, respectively. However, (*S*)–14-methyl epothilone B (epothilone B derivative 4) may be synthesized in a similar manner by substituting aldehyde 7 for aldehyde 6 in the first step of Scheme 2 of FIG. 3.

As shown in Scheme 2 of FIG. 3, intermolecular Ni/Cr coupling of vinyl iodide 8 with aldehyde 6 (2 equiv.) provided the intermediate allylic alcohol in 75% yield as a

mixture of diastereomers (1:1). Exposure of this mixture to thionyl chloride in ether-pentane provided the desired primary chloride in 85% yield. While the olefin geometry was completely selective a small amount of the secondary allylic chloride could be observed in the crude proton NMR spectrum. $LiEt_3BH$ not only reductively cleaved the chiral auxiliary but also efficiently reduced the allylic chloride in 72% yield providing primary alcohol 8c. Oxidation with Dess-Martin periodinane then provided aldehyde 9.

As highlighted in Scheme 3 of FIG. 4, the completion of the total synthesis began with an aldol reaction. The lithium enolate of ethyl ketone 10 was generated by exposure to LDA at -78 °C. Addition of aldehyde 9 provided the desired *syn, anti*-aldol adduct as the major product in 69% yield and 3.5:1 selectivity. After protection of the C7 hydroxyl as a TBS ether the primary alcohol was liberated by exposure to acidic methanol solution. The C1-carboxylic acid 11 was generated by two-step oxidation and subsequent selective removal of the C15 TBODPS (*t*-butoxydiphenylsilyl) ether was then accomplished with TBAF in a combined 71% yield. Macrolactonization proceeded efficiently using the Yamaguchi method[12] to provide the 16-membered lactone in 78% yield. Deprotection of the C3 and C7 silyl ethers was carried out using TFA providing the (*S*)-C14-methyl epothilone D 12. Finally, a highly selective incorporation of the C12-C13 epoxide was carried out by exposure to mCPBA yielding (*R*)-C14-methyl epothilone B in 55% yield.

Synthesis of (*R*)-C14-methyl epothilone D, epothilone D derivative 13 was accomplished via a similar synthetic sequence to that described for the synthesis of (*R*)-C14-methyl epothilone D. Epoxidation of this intermediate proceeded in a highly selective fashion as shown in Scheme 4 of FIG. 5. The conformational constraints imposed by the C14-methyl substituent makes it highly likely that the stereochemistry of the epoxide is epimeric to epothilone B and epothilone derivatives 3 and 4. The resulting epothilone derivative appears to have the structure shown at 14.

Single crystals of epothilone D derivative 12 and epothilone B derivative 3 were obtained by slow-evaporation techniques and x-ray diffraction studies unambiguously

determined their structure, Figure 2. In addition, the conformation of each in the solid-state was quite similar to that reported for epothilone B and thus confomer A. Proton NMR coupling constants ($J_{14\text{-}15}$ = 9.9, 10.5 Hz respectively) also supported this preference in solution. In contrast, proton NMR coupling constants of epothilone derivatives 13 and 14 had the expected values for conformer B ($J_{14\text{-}15}$ = 2.9, 1.2 Hz respectively).

Preliminary biological investigations of these compounds revealed significant tubulin polymerization activity for compounds 12 and 3. In contrast, the C14-diastereomeric compounds, epothilone derivatives 13 and 14, showed relatively weak activity. Similar profiles were observed in tumor cell growth assays. In fact, (*R*)-C14-methyl-epothilone B 3 was found to be >2x as active as epothilone B itself.

The conformation-activity relationships presented herein strongly support the importance of conformer A for the tubulin binding Moreover, this approach represents on new perspective on rational design of new chemotherapeutic agents.

The synthesis methods described above for epothilone derivatives 3 and 4 was highlighted by the efficient generation of a C12-C13 trisubstituted olefin which exploits a sequential Nozaki-Hiyama-Kishi coupling and a stereoselective thionyl chloride rearrangement. Using the above-described synthesis method, quantities of epothilone derivatives 3 and 4 may be produced.

<u>Example 2</u>

Materials and Methods

Cell lines and culture conditions: Human breast carcinoma cell line MCF-7, multi-drug resistant breast carcinoma cell line NCI/ADR, non-small cell lung carcinoma cell line NCI-H460 and glioma cell line SF-268 were obtained from the National Cancer Institute. All cell lines were maintained in RPMI-1640 medium (Gibco/BRL, Rockville,

MD) supplemented with 2 mM L-glutamine, 25 mM HEPES and 10% FBS (Hyclone, Logan, UT). Cells were maintained in a humidified incubator at 37°C in 5% CO_2.

Cytotoxicity assays: Tumor cells were seeded in 100 µl at 5000 (MCF-7), 7500 (NCI/ADR), 5000 (NCI-H460) and 7500 (SF-268) cells per well in 96-well plates. Cells were allowed to adhere for 24 hours. Each compound ranging from 0.001 to 1000 nM in 100 µl was added to cells in triplicate wells. After 3 days, cells were fixed at 4°C for 1 hour with 10% trichloroacetic acid and then stained with 0.2% sulforhodamine B (SRB)/1% acetic acid for 20 minutes at room temperature. The unbounded dye was rinsed away with 1% acetic acid, and the bounded SRB was then extracted with 200 µl of 10 mM Tris base. The absorbance was measured at 515 nm using a 96-well microtiter plate reader (Spectra Max 250, Molecular Devices). The IC_{50} values were calculated using a KaleidaGraph program. The experiments were performed twice.

Results

IC_{50} (nM) Values

Compound	MCF-7	NCI-ADR	H460	SF
Epothilone D	5	26	20	7
14s-methyl Epothilone D	35	238	42	42
14r-methyl Epothilone B	3	23	3	3
14r-methyl Epothilone D	>1000	>1000	>1000	>1000
14s-methyl Epothilone B	>1000	>1000	>1000	>1000

Although the present invention has been fully described in conjunction with the preferred embodiment thereof with reference to the accompanying drawings, it is to be understood that various changes and modifications may be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present invention as defined by the appended claims, unless they depart therefrom.

WHAT IS CLAIMED IS:

1. A compound comprising (*R*)-C14-methyl-epothilone B.

2. A compound comprising (*S*)-C14-methyl-epothilone B.

3. A compound comprising (*S*)-C14-methyl-epothilone D.

4. A compound comprising (*R*)-C14-methyl-epothilone D.

5. A method for synthesizing (*R*)-C14-methyl-epothilone B comprising the steps of:

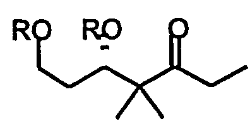
(a) providing (*S*)-C14-methyl-epothilone D; and

(b) incorporating an epoxide group at the C12-C14 position of (*S*)-C14-methyl-epothilone D to form (*R*)-C14-methyl-epothilone B.

6. The method of claim 5, wherein step (b) is carried out by exposing (*S*)-C14-methyl-epothilone D to mCPBA.

7. The method of claim 5, wherein (*S*)-C14-methyl-epothilone D is provided synthesizing (*S*)-C14-methyl-epothilone D by a method comprising the following steps:

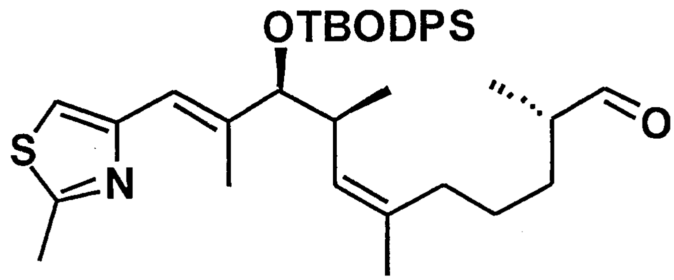
(c) providing an ethyl ketone 10 having the following formula:



;

(d) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

(e) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



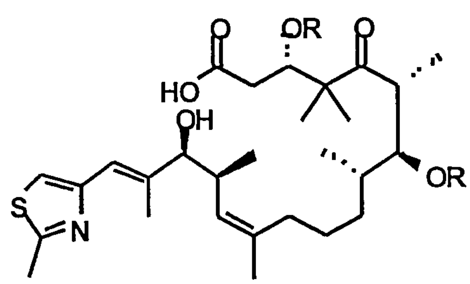
to thereby form a *syn.*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl;

(f) exposing said *syn.*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether;

(g) de-protecting said *syn.*, *anti*-aldol adduct to form a primary alcohol;

(h) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid;

(i) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:



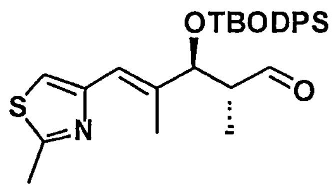
;

(j) macrolactonizing compound 11 to form a 16-membered lactone; and

(k) treating said 16-membered lactone with TFA to form (*S*)-C14-methyl-epothilone D.

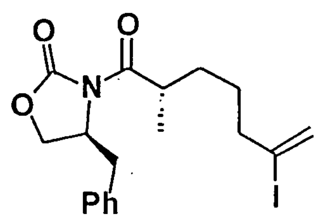
7. The method of claim 6, wherein aldehyde 9 is synthesized by a method comprising the following steps:

(l) providing aldehyde 6 having the following formula:



;

(m) intermolecularly coupling aldehyde 6 with a vinyl iodide 8 having the following formula:



using Ni/Cr coupling to thereby form an intermediate allylic alcohol;

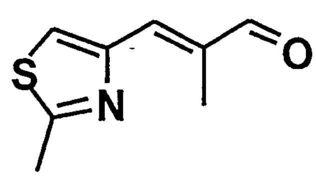
(n) reacting said allylic alcohol thionyl chloride to produce a primary allylic chloride;

(o) reacting said primary allylic chloride with $LiEt_3BH$ to form a primary allylic alcohol; and

(p) oxidizing said primary allylic alcohol to form aldehyde 9.

8. The method of claim 7, wherein aldehyde 6 is provided by synthesizing aldehyde 6 using a method comprising the following steps:

(q) providing thiazole aldehyde 5 having the following formula:



;

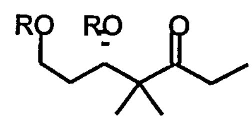
(r) reacting thiazole aldehyde 5 with Z-crotyl(+)-Ipc_2B to form a homoallylic alcohol compound;

(s) reacting said homoallylic alcohol with TBODPSCl to form an etherized compound; and

(t) oxidatively cleaving a terminal alkene of said etherized compound to form aldehyde 6.

9. A method for synthesizing (*S*)-C14-methyl-epothilone D comprising the following steps:

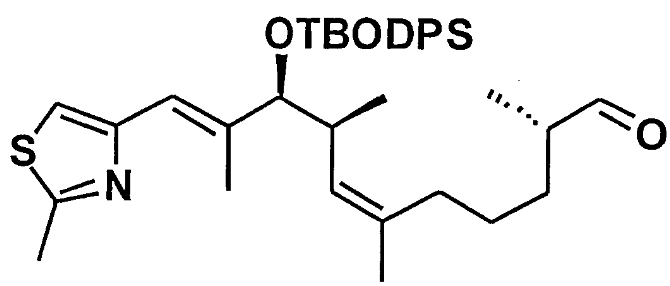
(a) providing an ethyl ketone 10 having the following formula:



;

(b) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

(c) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



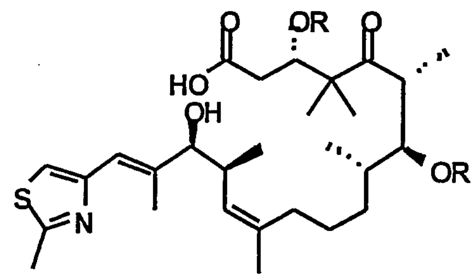
to thereby form a *syn.*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl;

(d) exposing said *syn.*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether;

(e) de-protecting said *syn.*, *anti*-aldol adduct to form a primary alcohol;

(f) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid;

(g) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:



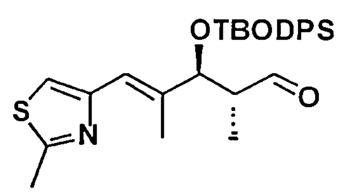
;

(h) macrolactonizing compound 11 to form a 16-membered lactone; and

(i) treating said 16-membered lactone with TFA to form (*S*)-C14-methyl-epothilone D.

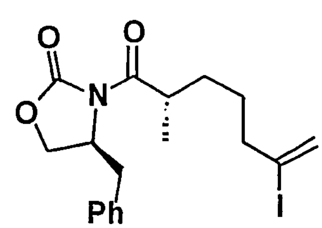
10. The method of claim 9, wherein aldehyde 9 is synthesized by a method comprising the following steps:

(j) providing aldehyde 6 having the following formula:



;

(k) intermolecularly coupling aldehyde 6 with a vinyl iodide 8 having the following formula:



using Ni/Cr coupling to thereby form an intermediate allylic alcohol;

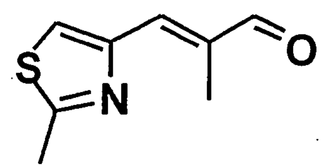
(l) reacting said allylic alcohol thionyl chloride to produce a primary allylic chloride;

(m) reacting said primary allylic chloride with $LiEt_3BH$ to form a primary allylic alcohol; and

(n) oxidizing said primary allylic alcohol to form aldehyde 9.

11. The method of claim 10, wherein aldehyde 6 is provided by synthesizing aldehyde 6 using a method comprising the following steps:

(o) providing thiazole aldehyde 5 having the following formula:



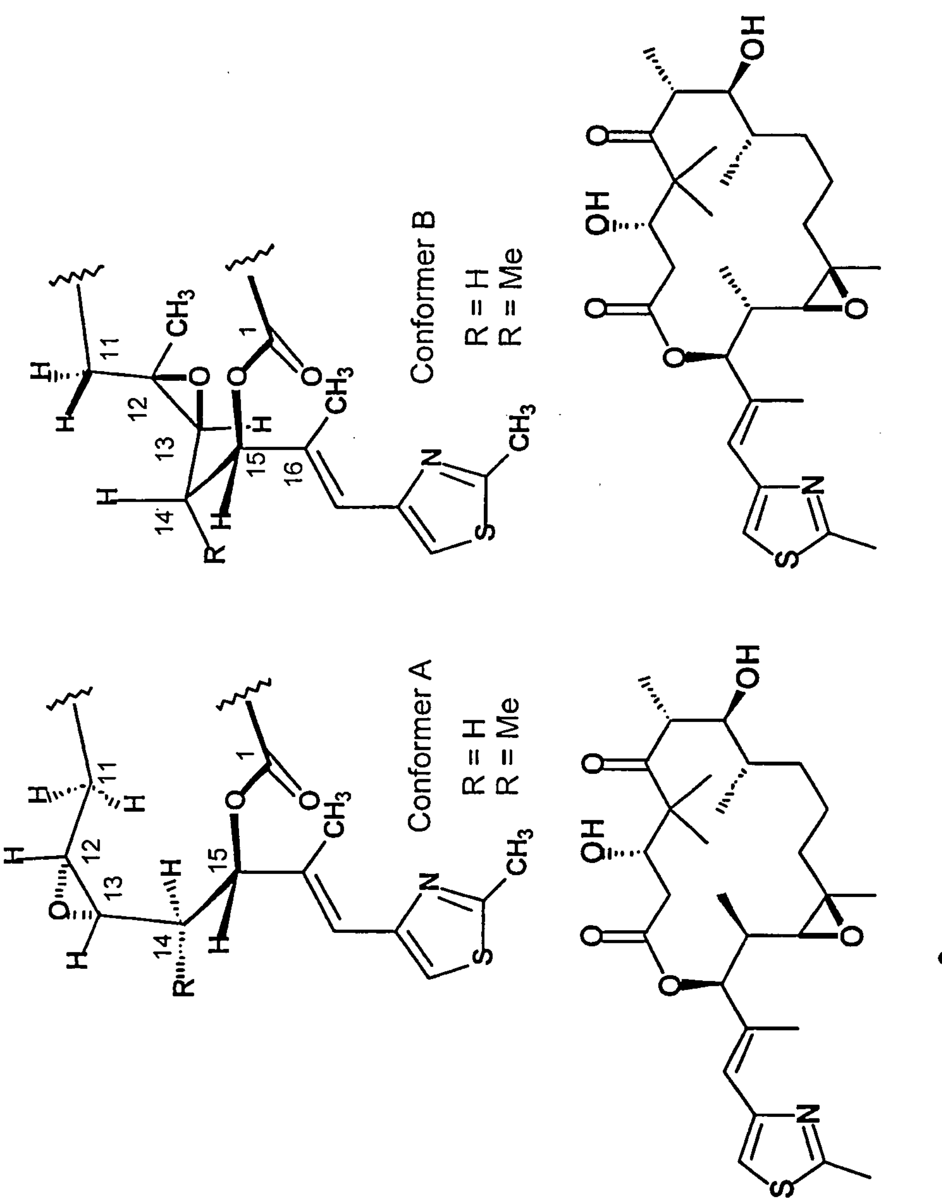
;

(q) reacting thiazole aldehyde 5 with Z-crotyl(+)-Ipc_2B to form a chloroborated compound;

(r) reacting said chloroborated aldehyde with TBODPSCl to form an etherized compound; and

(s) oxidatively cleaving a terminal alkene of said etherized compound to form aldehyde 6.

FIG. 1



3

4

FIG. 2

Scheme 1

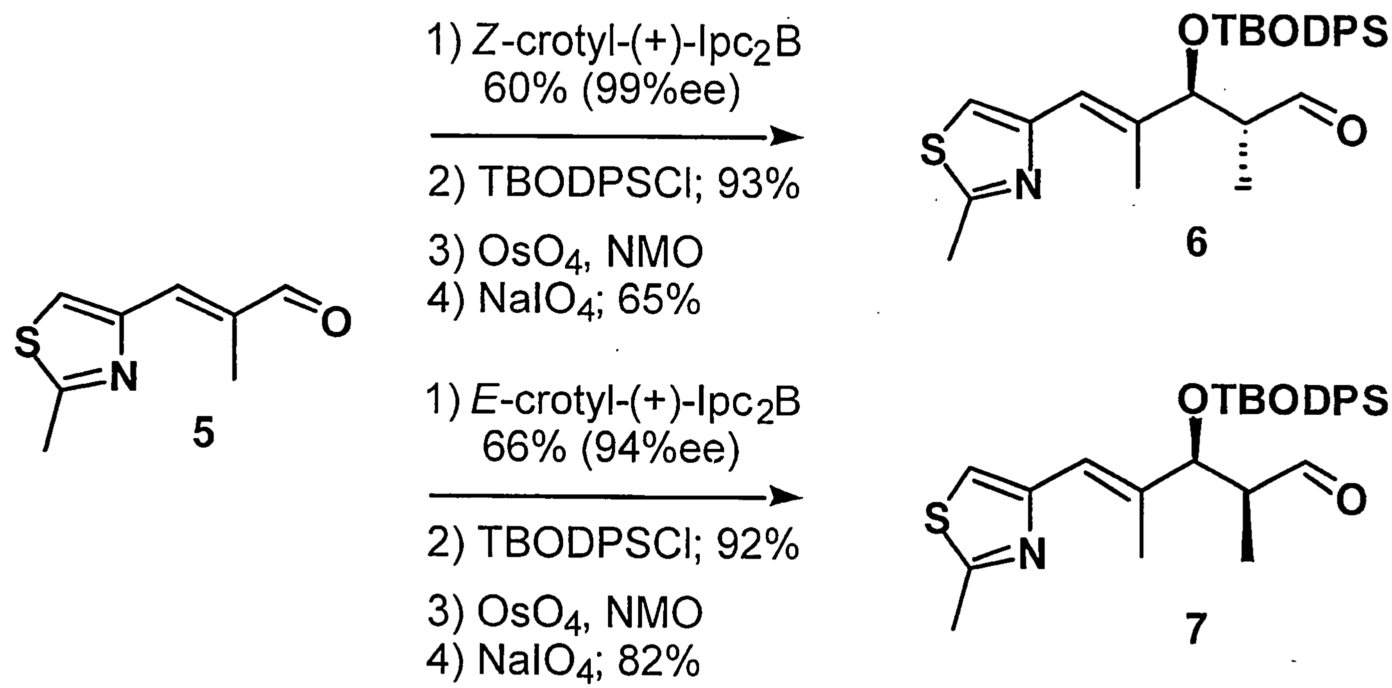


FIG. 3

Scheme 2

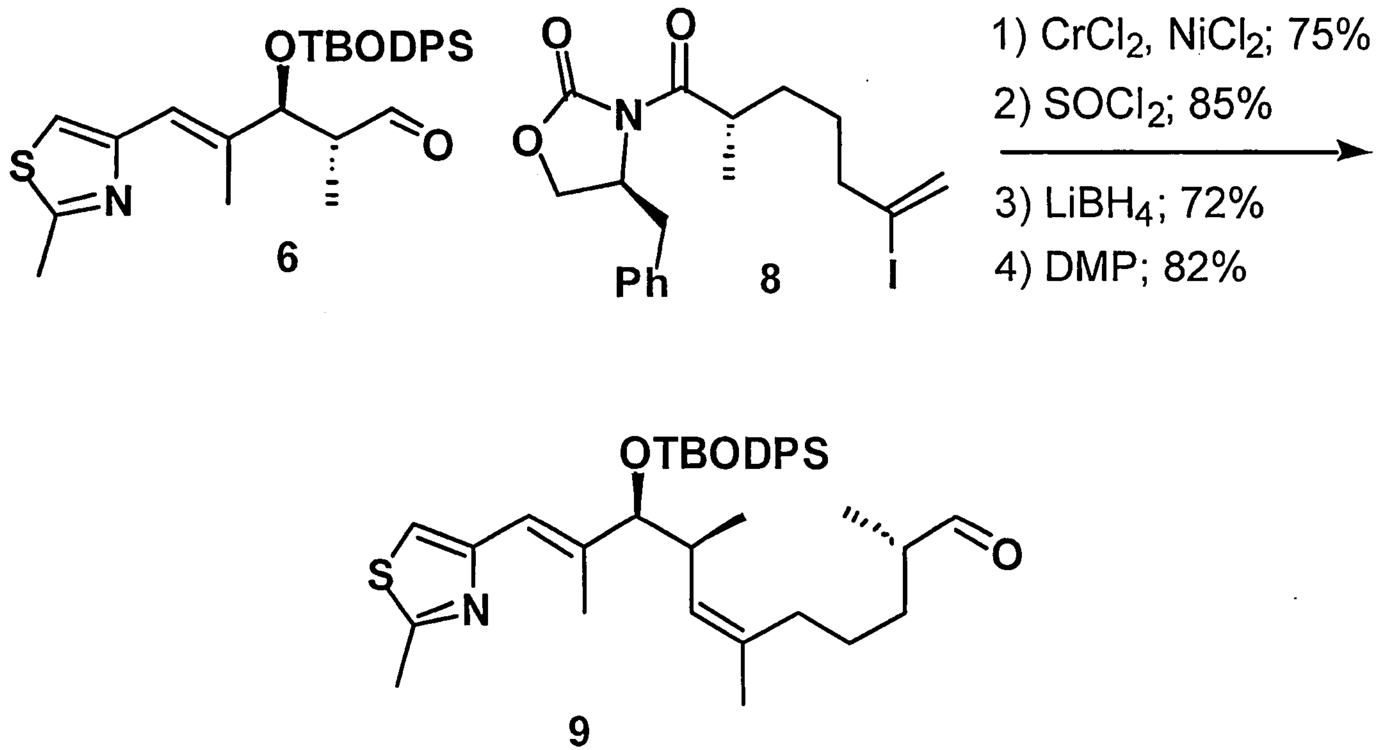
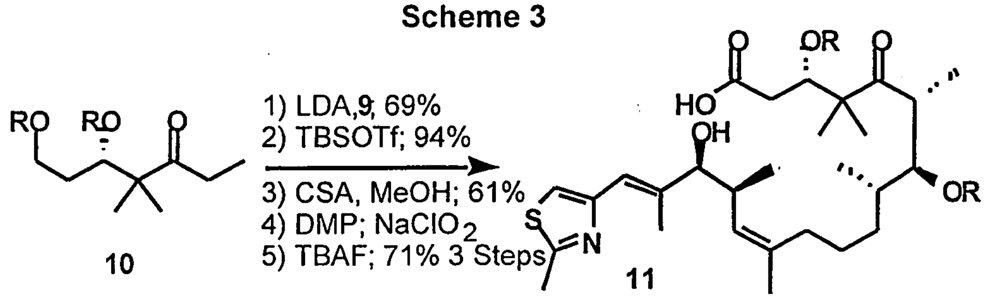
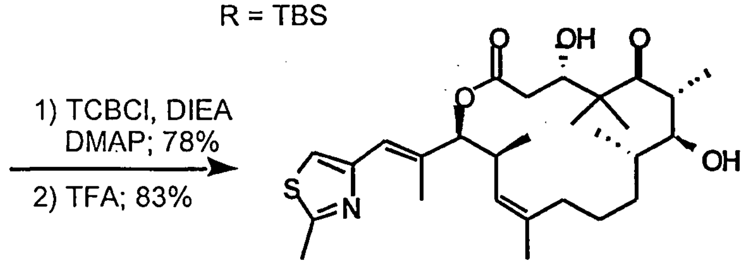


FIG. 4

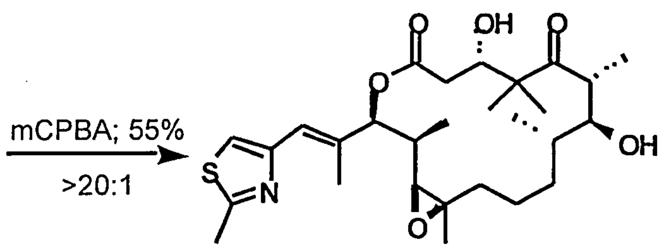
Scheme 3



R = TBS



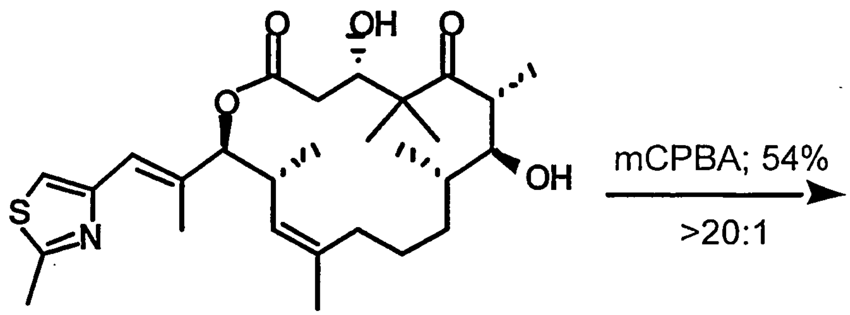
12 (*S*) -14-methyl-epothilone D



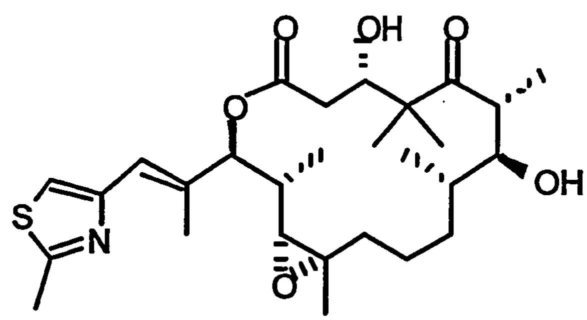
3 (*R*) -14-methyl-epothilone B

FIG. 5

Scheme 4



13 (*R*) -14-methyl-epothilone D



14 (*S*) -14-methyl analogue

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/06113

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : ~~C07D 417/06~~
US CL : 548/204

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 548/204

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/083800 A2 (KOSAN BIOSCIENCES, INC.) 8 November 2001 (08.11.2001), see example 11G.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 May 2003 (19.05.2003)	02 JUN 2003

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Felicia D. Roberts for Robert Gerstl Telephone No. 703 308-1235

US 20030176368A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2003/0176368 A1
Danishefsky et al. (43) Pub. Date: Sep. 18, 2003

(54) SYNTHESIS OF EPOTHILONES, INTERMEDIATES THERETO AND ANALOGUES THEREOF

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(21) Appl. No.: 10/236,135

(22) Filed: Sep. 6, 2002

Related U.S. Application Data

(60) Provisional application No. 60/317,637, filed on Sep. 6, 2001. Provisional application No. 60/351,576, filed on Oct. 26, 2001.

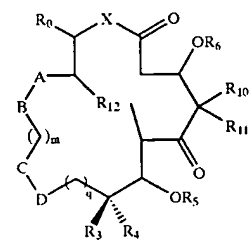
Publication Classification

(51) Int. Cl.[7] C07H 17/08; C07D 49/02; A61K 31/7048; A61K 31/427; A61K 31/38; A61K 31/365; C07D 417/02

(52) U.S. Cl. 514/28; 514/365; 514/183; 514/431; 514/450; 536/7.4; 548/203; 540/451; 549/9; 549/266

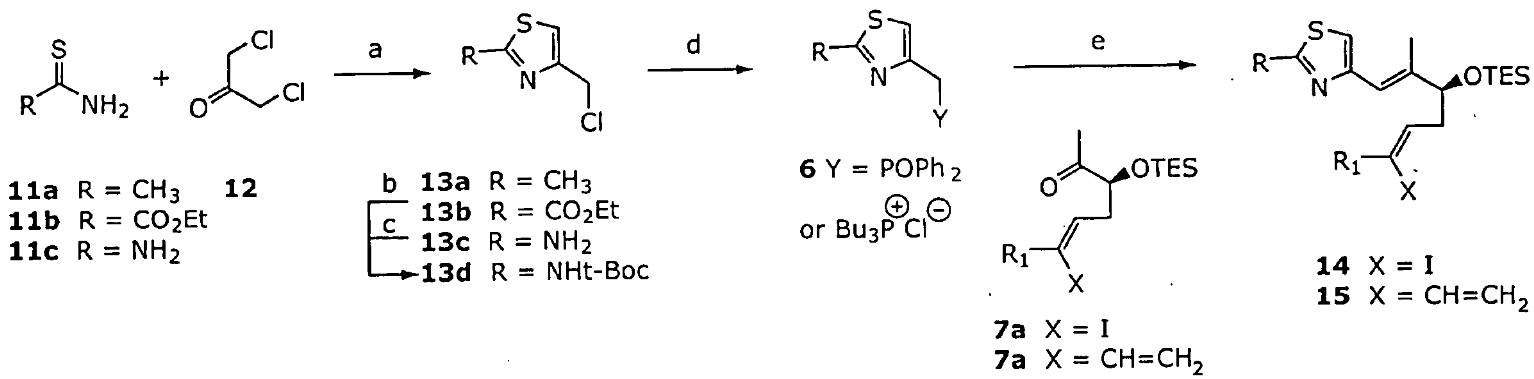
(57) ABSTRACT

The present invention provides compounds of formula (I):



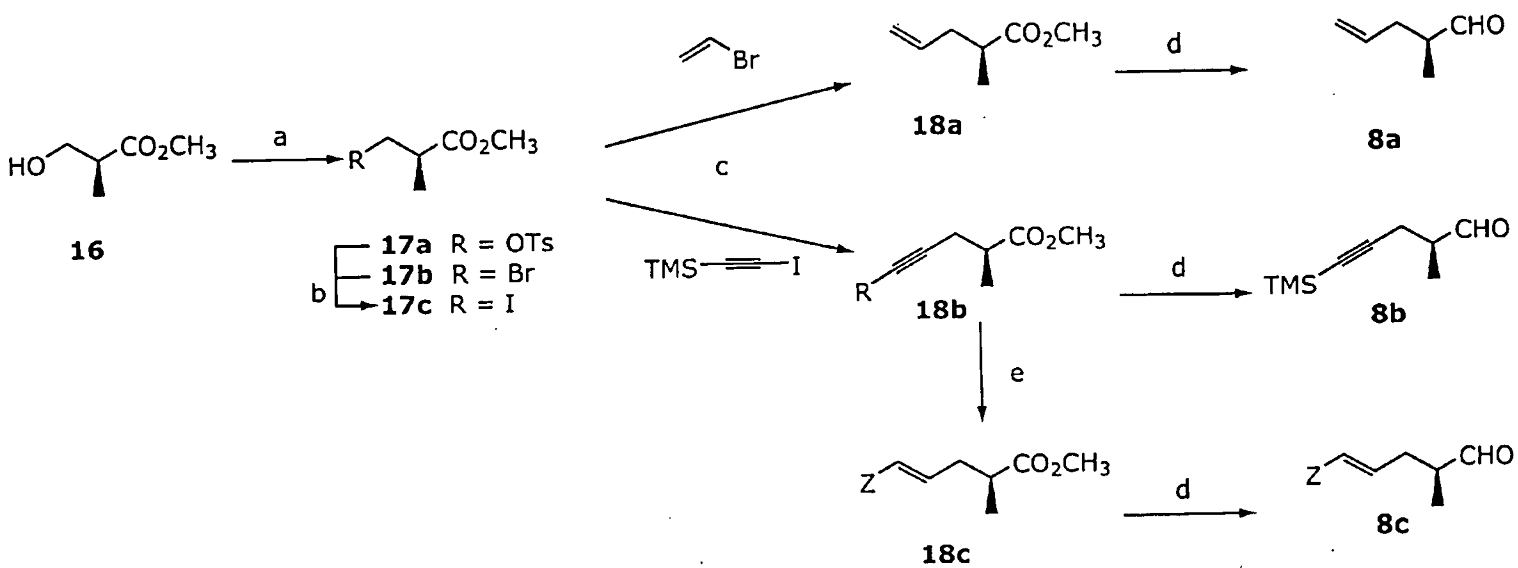
(I)

as described generally and in classes and subclasses herein. The present invention additionally provides pharmaceutical compositions comprising compounds of formula (I) and provides methods of treating cancer comprising administering a compound of formula (I).



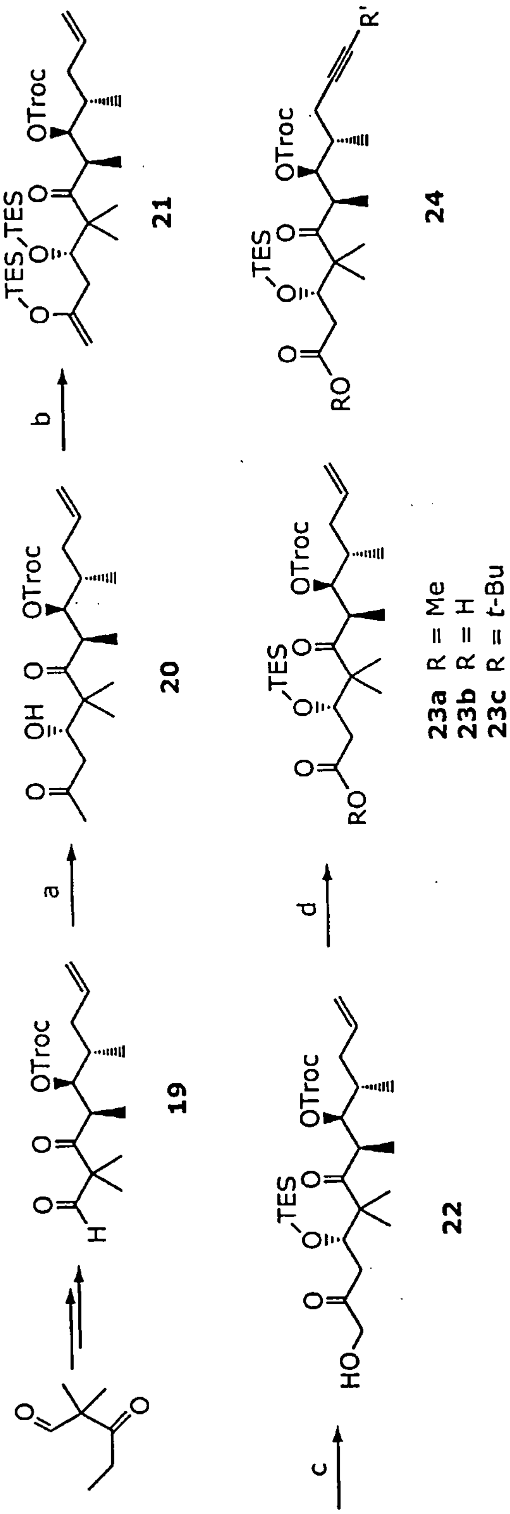
(a) reflux; (b) i) LiOH, aq. THF, ii) $N_3PO(OR)_2$, iii) t-BuOH, reflux; (c) $(t\text{-Boc})_2O$, THF; (d) Cs_2CO_3, $HOPPh_2$ or PBu_3; (e) LiHMDS, THF

FIG. 1



(a) TsCl, Pyr., PPh_3, Br_2 or PPh_3, I_2, Imidazole; (b) NaI, DMF; (c) Zn, TMSCl, DMF then Pd_2dba_3, $P(o\text{-}Tol)_3$; (d) Dibal-H, CH_2Cl_2; (e) i) TBAF/THF, then A) $RhCl(PPh_3)_3$, catecol borane, B) Bu_3SnH, or C) $ArRuCl(PPh_3)_2$, $HSiR_3$.

FIG. 2



(a) Acetone, cat. D-proline, rt; (b) i) TESOTf, lutidine, CH_2Cl_2 ii) TMSOTf; (c) DMDO, CH_2Cl_2; (d) $Pb(OAc)_4$, Benzene/MeOH or Benzene.

FIG. 3

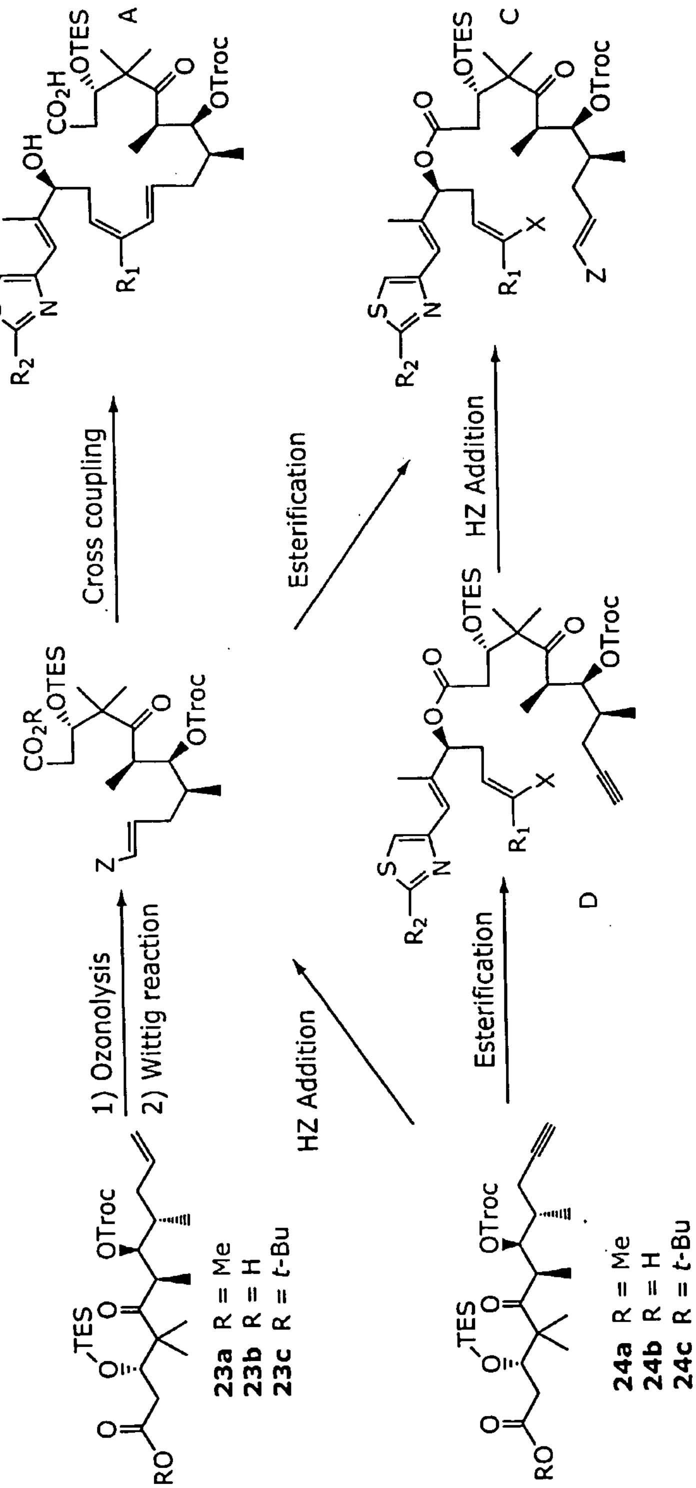

23a R = Me
23b R = H
23c R = t-Bu
24a R = Me
24b R = H
24c R = t-Bu
1) Ozonolysis
2) Wittig reaction
HZ Addition
Esterification
Cross coupling
Esterification
HZ Addition
A
C
D

FIG. 4

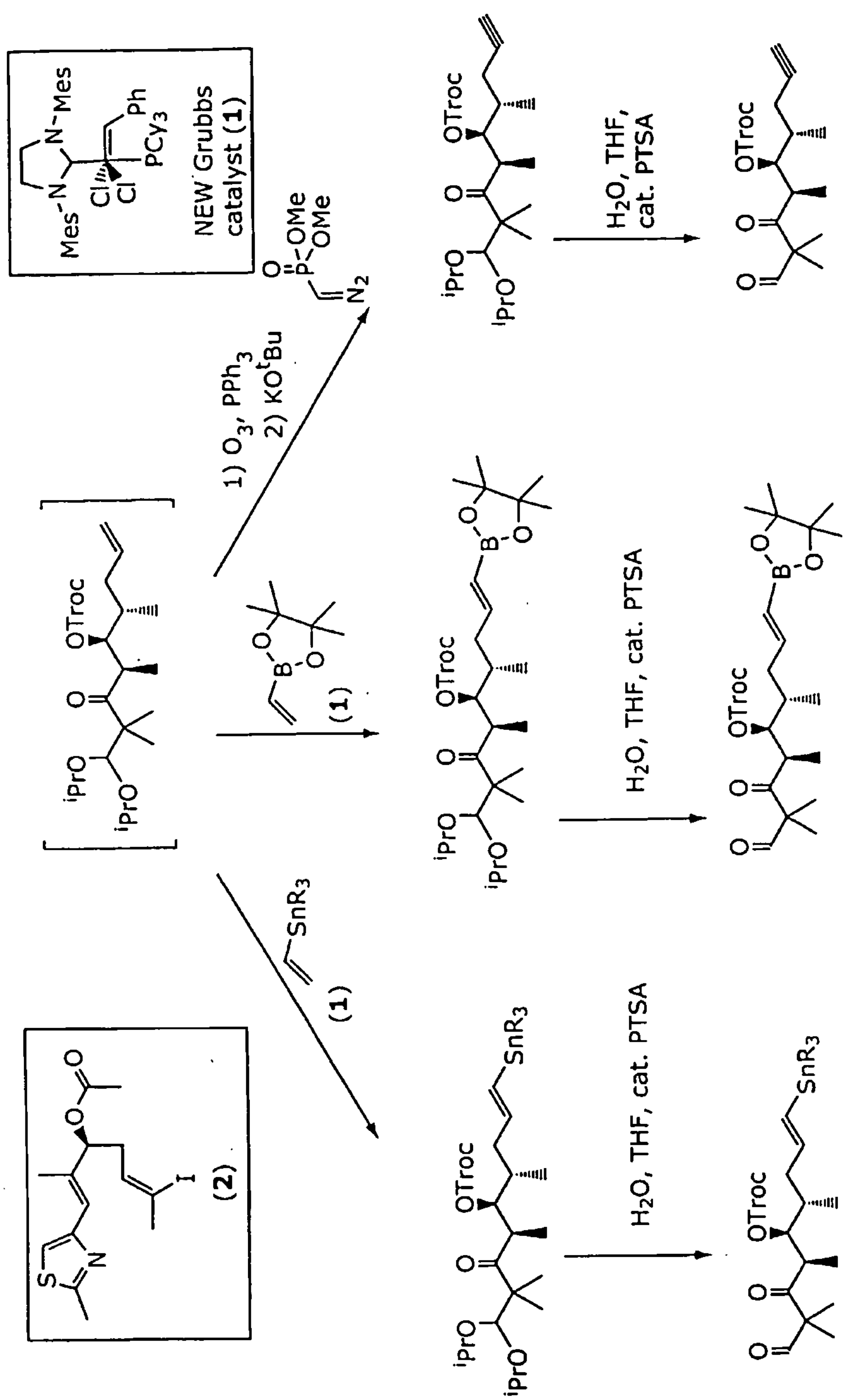

Mes
N
N
Mes
Cl
Cl
Ru
Ph
PCy3
NEW Grubbs catalyst (1)
(2)
1) O3, PPh3
2) KOtBu
(1)
(1)
H2O, THF, cat. PTSA
SnR3
OTroc
iPrO

FIG. 5A

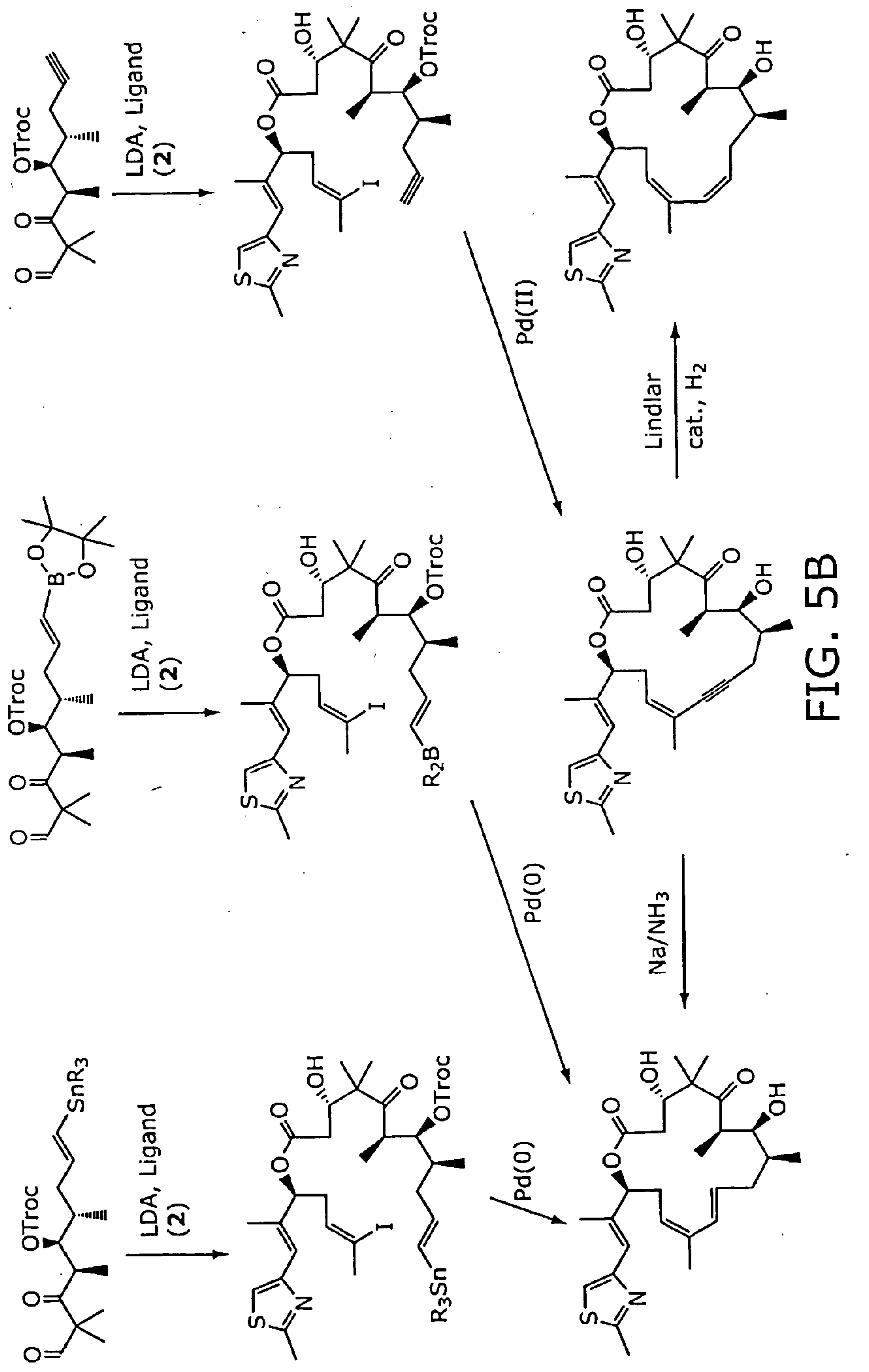


FIG. 5B

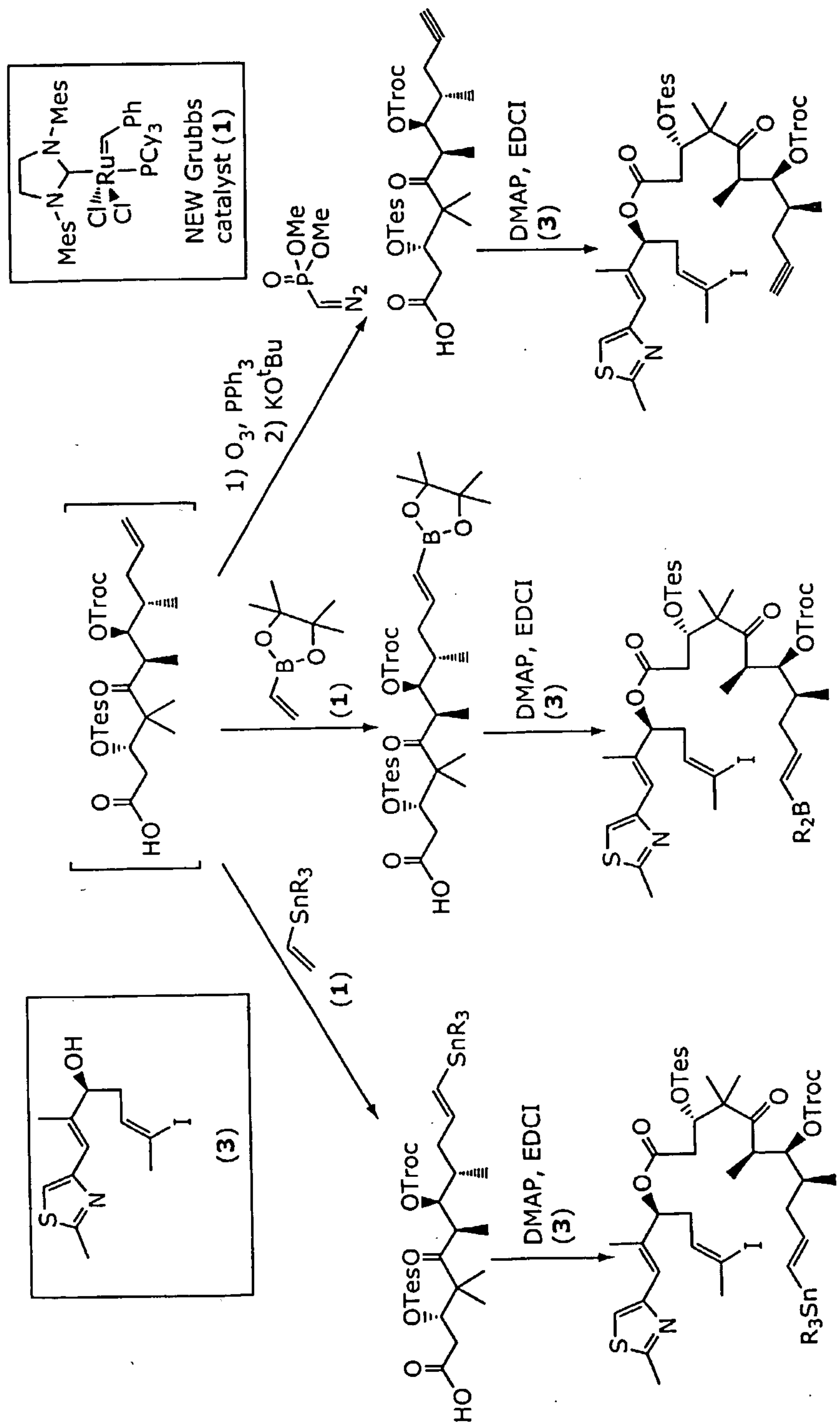


FIG. 6A

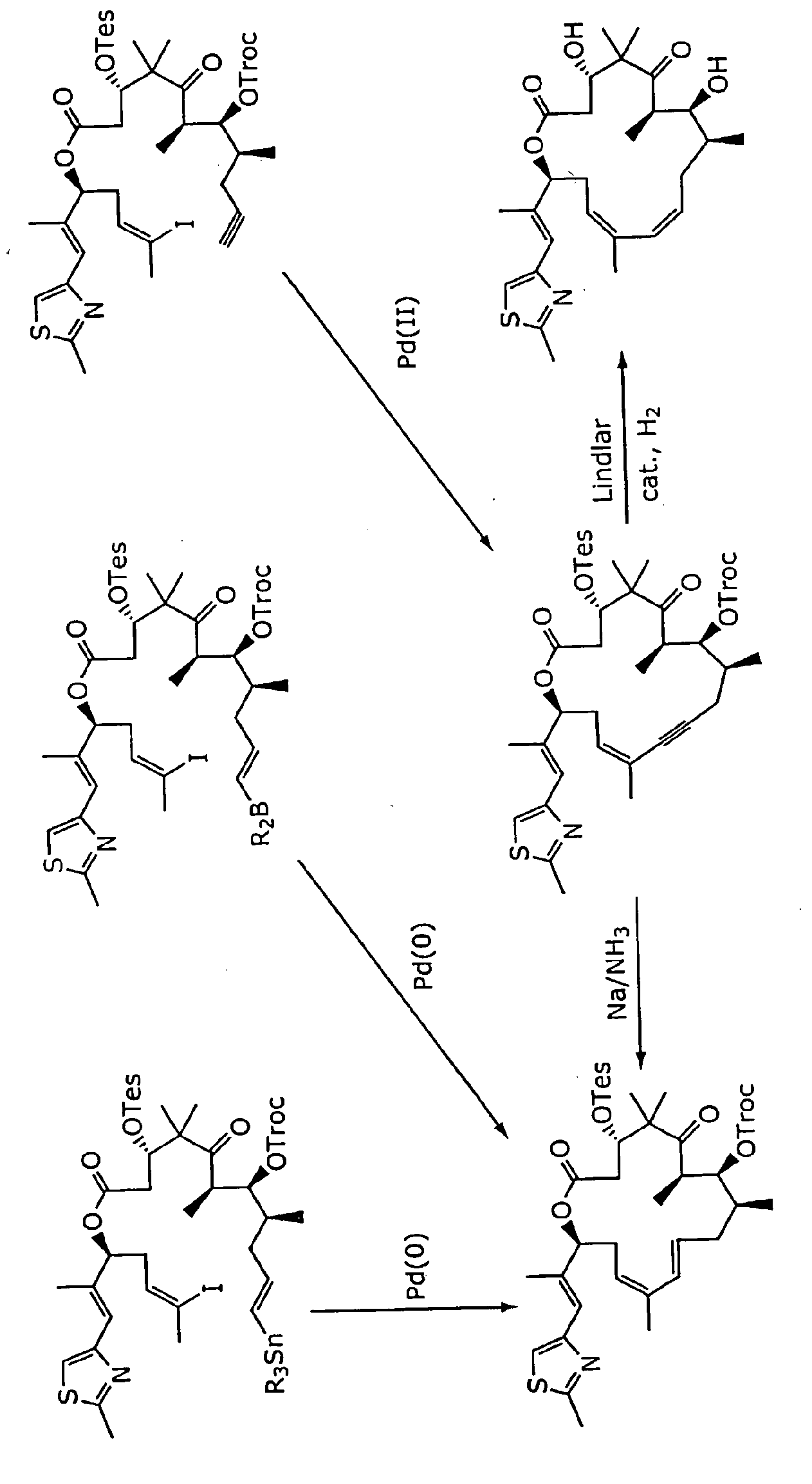


FIG. 6B

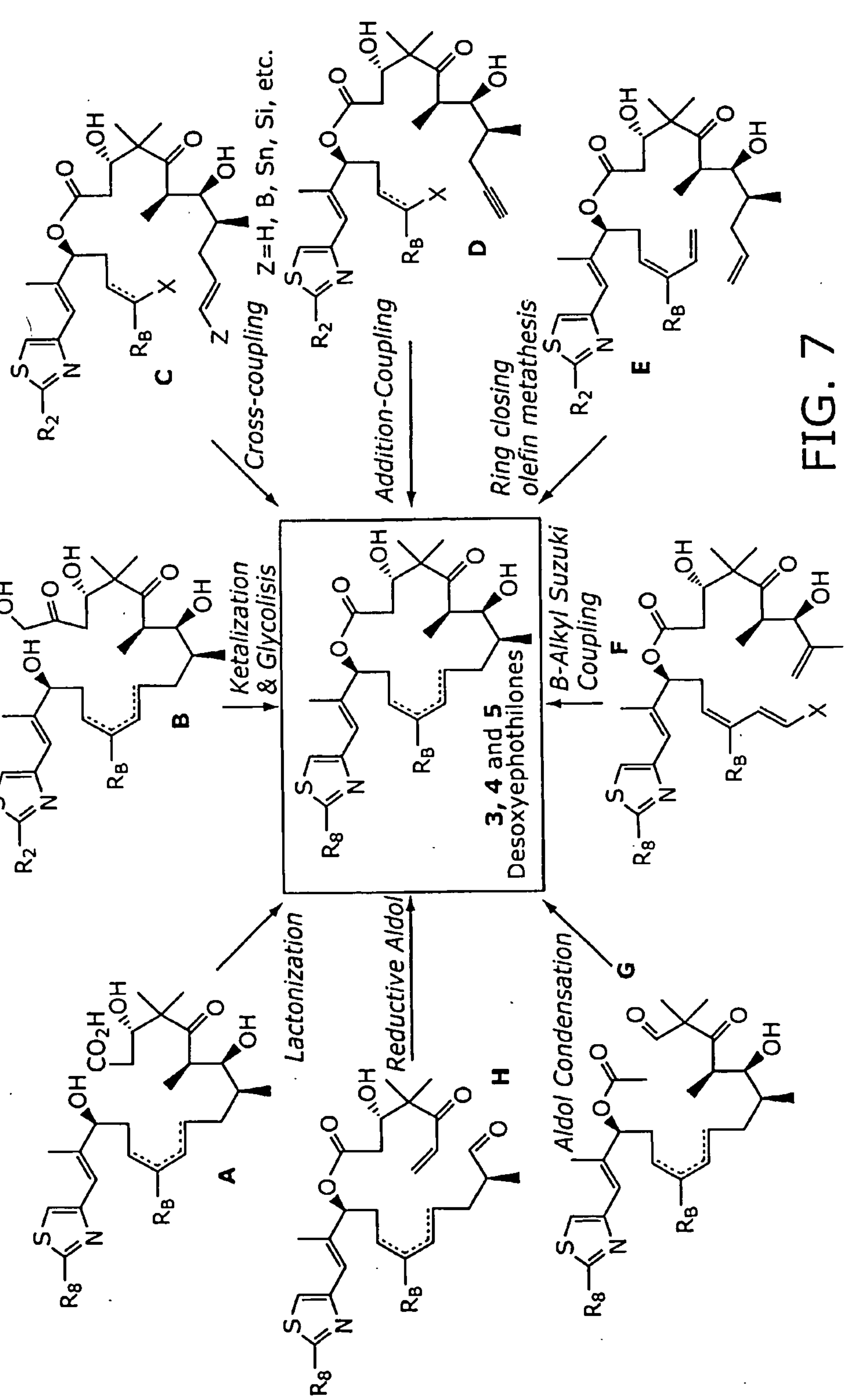

Cross-coupling
Z=H, B, Sn, Si, etc.
Addition-Coupling
Ring closing olefin metathesis
Ketalization & Glycolisis
B-Alkyl Suzuki Coupling
3, 4 and 5
Desoxyepothilones
Lactonization
Reductive Aldol
Aldol Condensation
A
B
C
D
E
F
G
H

FIG. 7

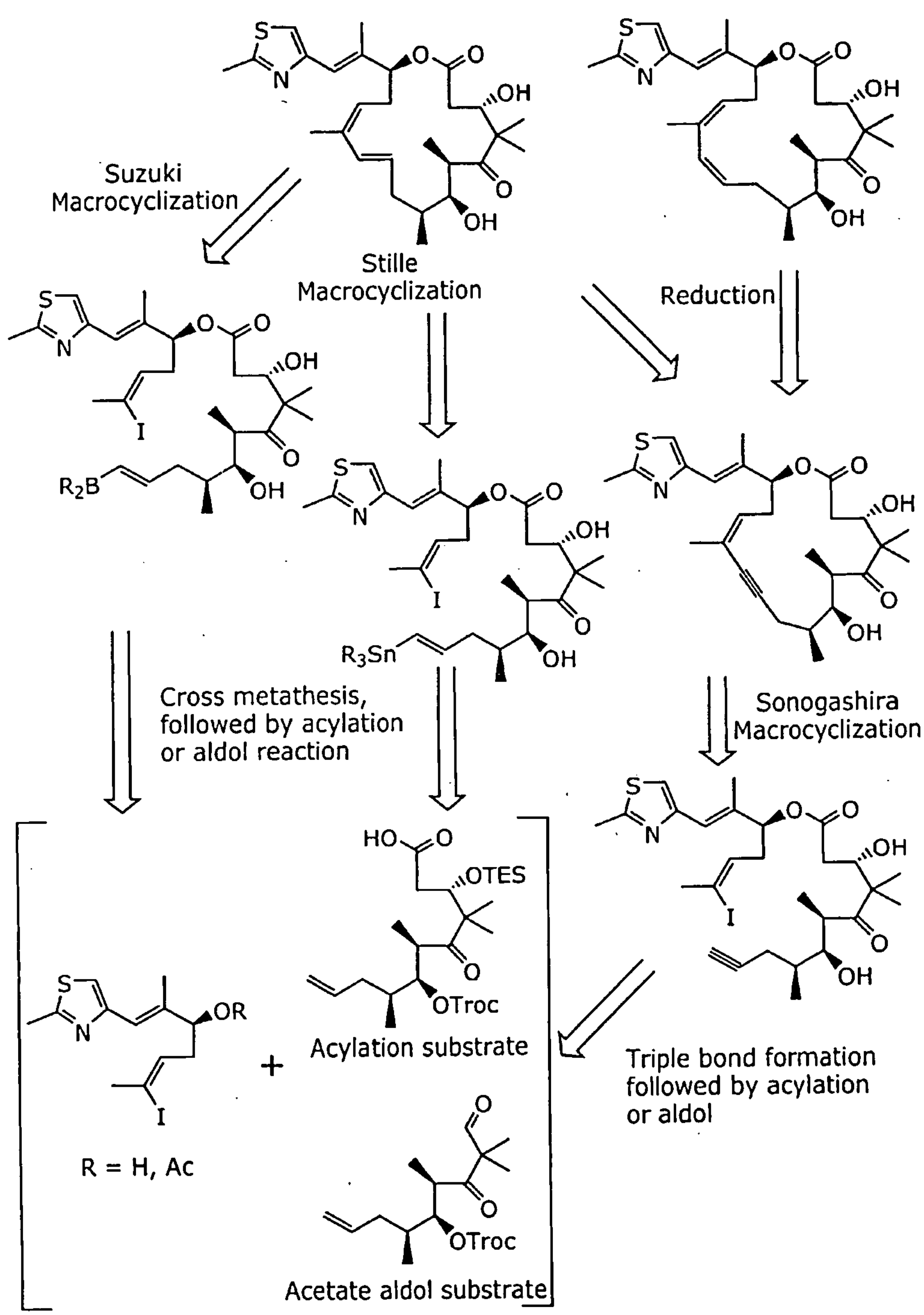

Suzuki
Macrocyclization
Stille
Macrocyclization
Reduction
Cross metathesis, followed by acylation or aldol reaction
Sonogashira
Macrocyclization
R = H, Ac
Acylation substrate
Acetate aldol substrate
Triple bond formation followed by acylation or aldol

FIG. 8

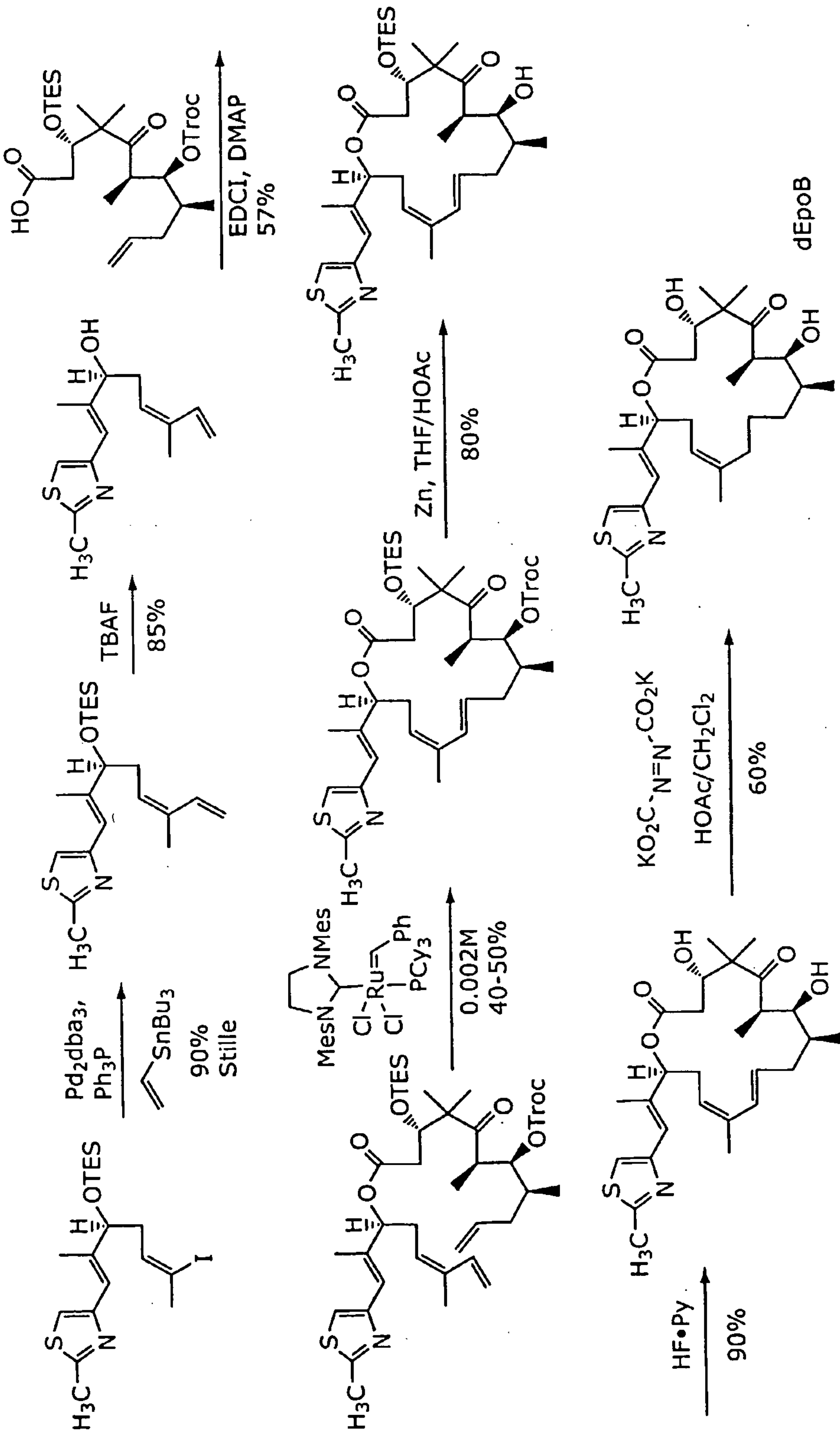

OTES
I
Pd2dba3, Ph3P
SnBu3
90% Stille
TBAF
85%
OH
HO
OTroc
EDCI, DMAP
57%
MesN
NMes
Cl
Ru
Ph
PCy3
0.002M
40-50%
Zn, THF/HOAc
80%
HF•Py
90%
KO2C-N=N-CO2K
HOAc/CH2Cl2
60%
dEpoB
H3C
S
N

FIG. 9

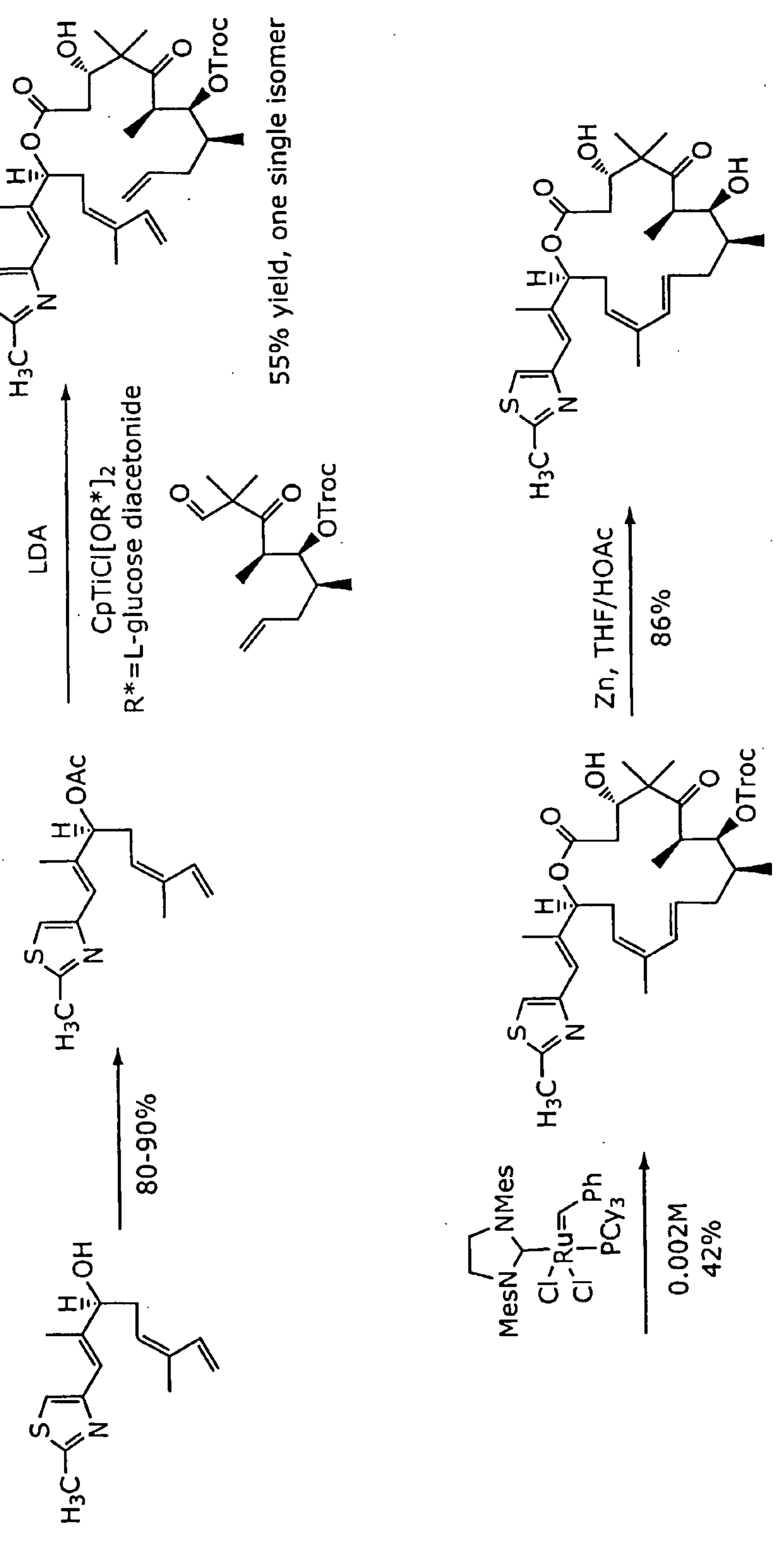

80-90%
LDA
CpTiCl[OR*]2
R*=L-glucose diacetonide
55% yield, one single isomer
0.002M
42%
Zn, THF/HOAc
86%

FIG. 10

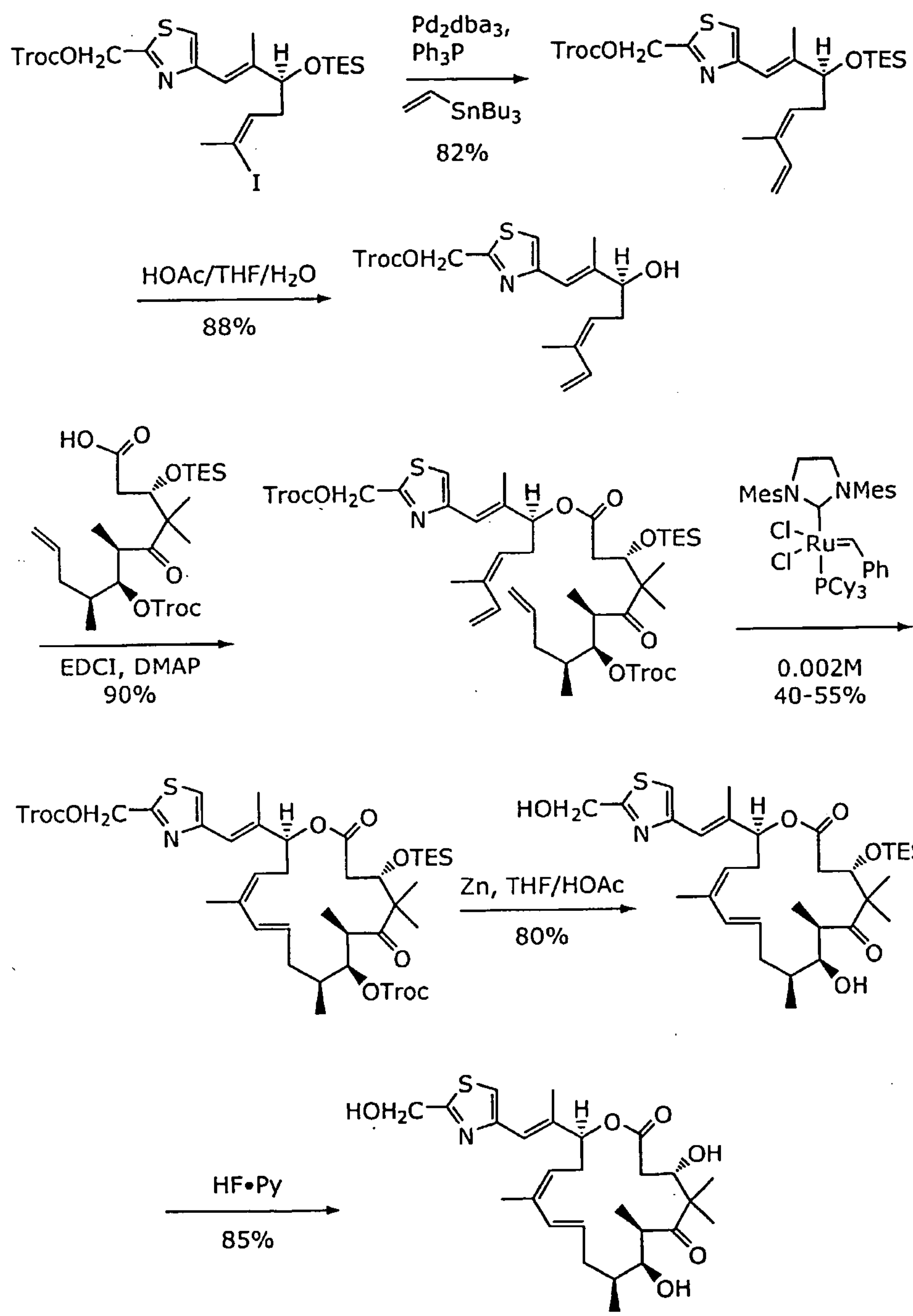

TrocOH2C
S
N
H
OTES
I
Pd2dba3,
Ph3P
SnBu3
82%
HOAc/THF/H2O
88%
OH
HO
O
OTroc
EDCI, DMAP
90%
MesN
NMes
Cl
Ru
Ph
PCy3
0.002M
40-55%
Zn, THF/HOAc
80%
HOH2C
HF•Py
85%

FIG. 11

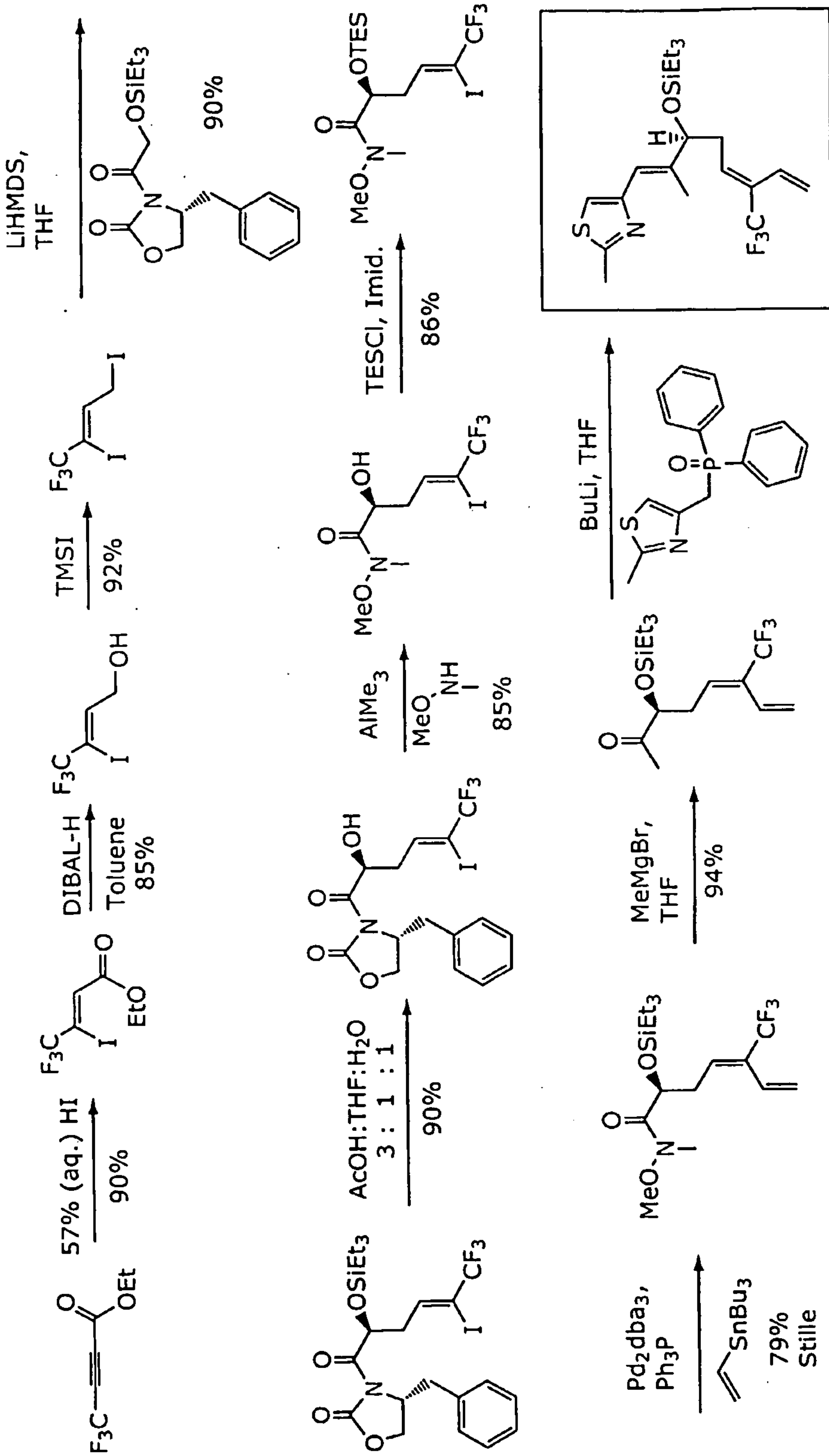

57% (aq.) HI
90%
DIBAL-H
Toluene
85%
TMSI
92%
LiHMDS,
THF
90%
AcOH:THF:H2O
3 : 1 : 1
90%
AlMe3
85%
TESCl, Imid.
86%
Pd2dba3,
Ph3P
SnBu3
79%
Stille
MeMgBr,
THF
94%
BuLi, THF
81%

FIG. 12A

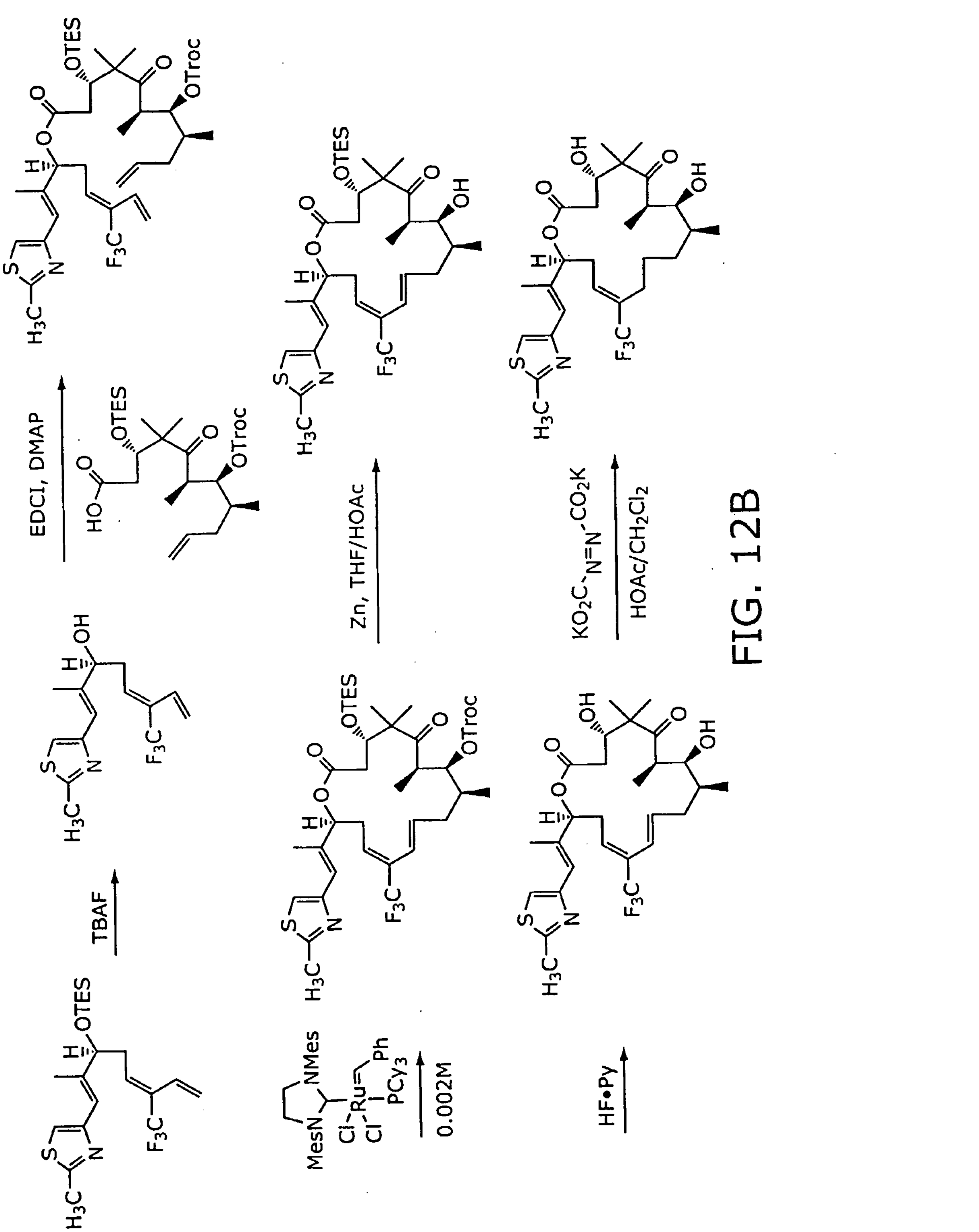

TBAF
EDCI, DMAP
0.002M
Zn, THF/HOAc
HF•Py
KO2C-N=N-CO2K
HOAc/CH2Cl2

FIG. 12B

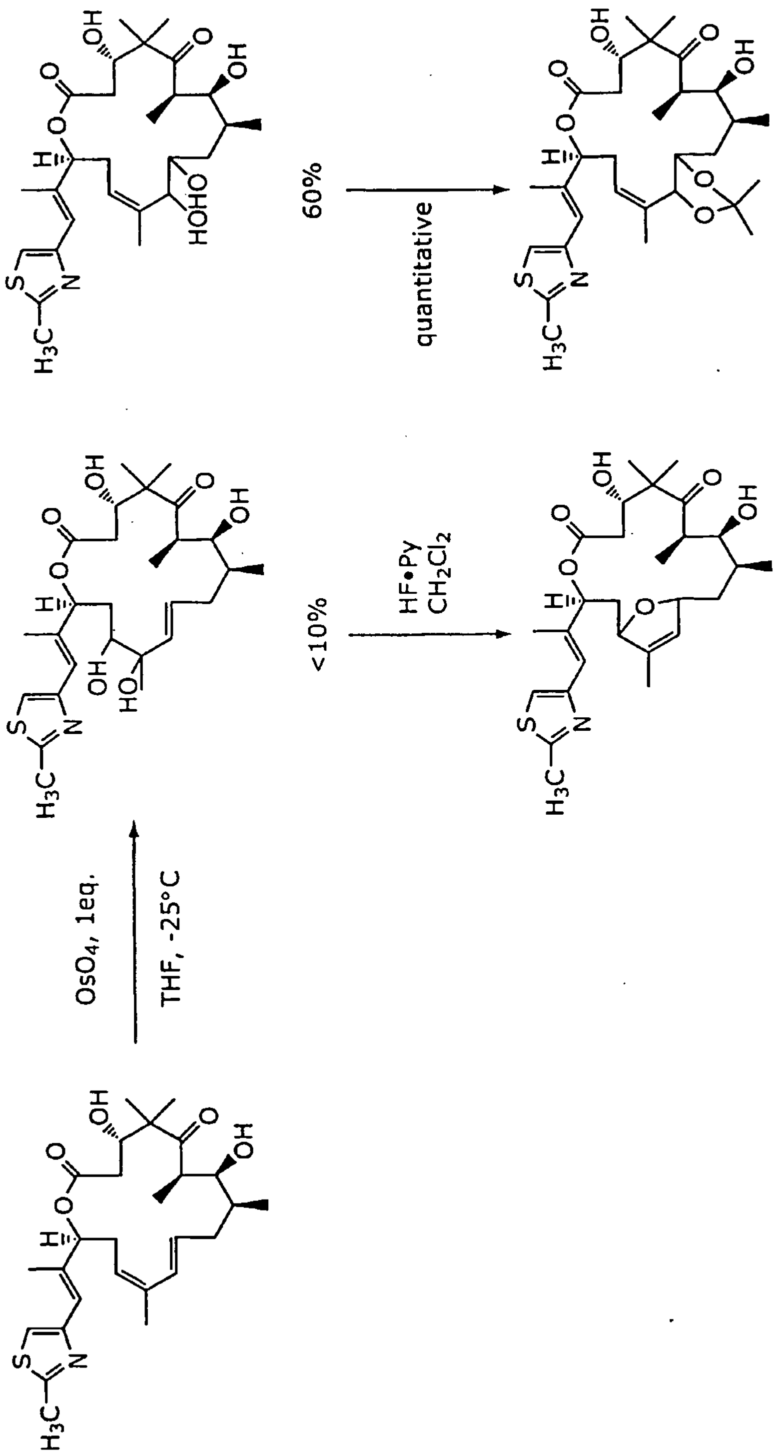


FIG. 13

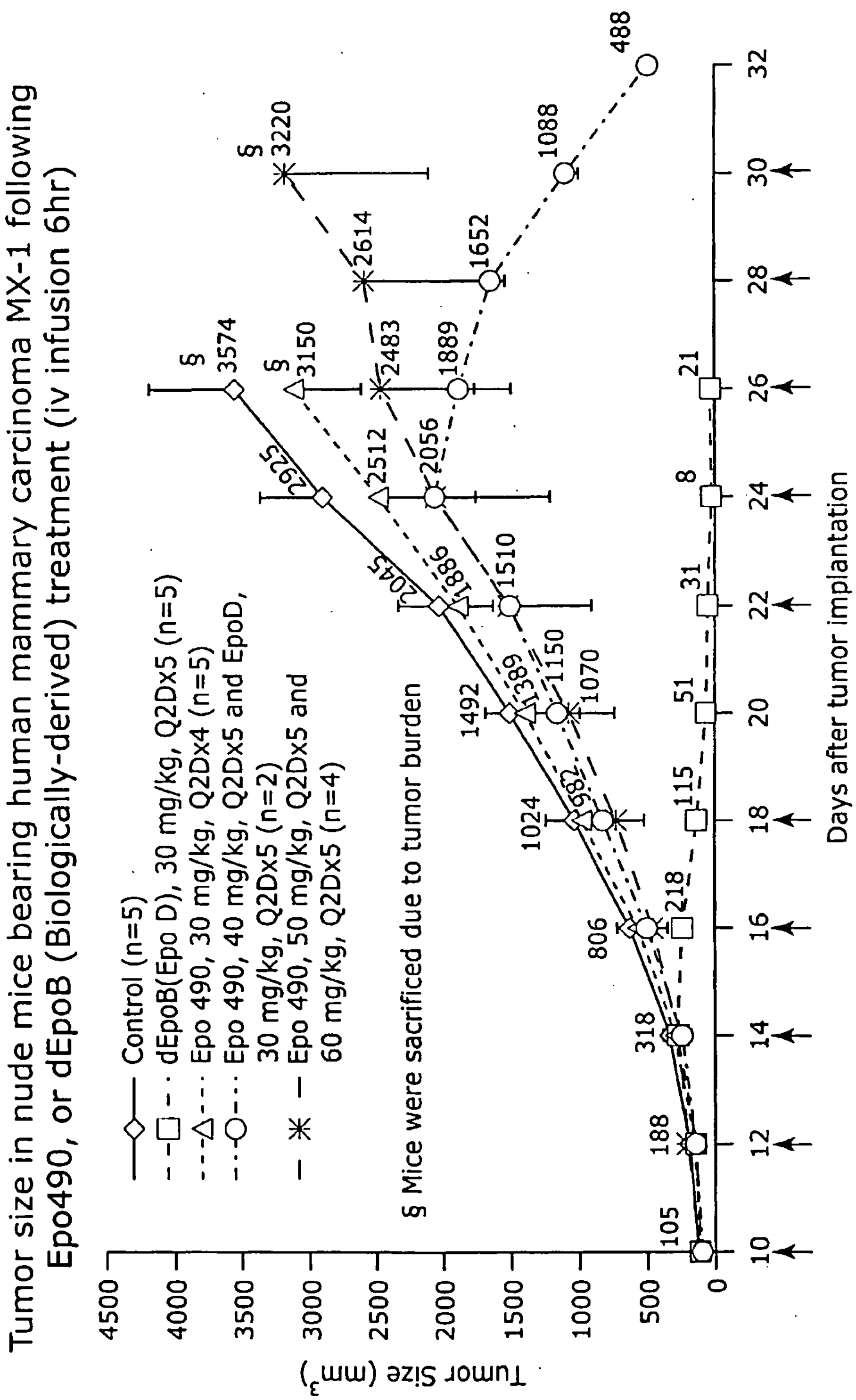

Tumor size in nude mice bearing human mammary carcinoma MX-1 following Epo490, or dEpoB (Biologically-derived) treatment (iv infusion 6hr)
Control (n=5)
dEpoB(Epo D), 30 mg/kg, Q2Dx5 (n=5)
Epo 490, 30 mg/kg, Q2Dx4 (n=5)
Epo 490, 40 mg/kg, Q2Dx5 and EpoD, 30 mg/kg, Q2Dx5 (n=2)
Epo 490, 50 mg/kg, Q2Dx5 and 60 mg/kg, Q2Dx5 (n=4)
§ Mice were sacrificed due to tumor burden
Tumor Size (mm³)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
Days after tumor implantation
10
12
14
16
18
20
22
24
26
28
30
32
105
188
318
806
218
1024
982
115
1492
1389
1150
1070
51
2045
1886
1510
31
2925
2512
2056
8
3574
3150
2483
1889
21
2614
1652
3220
1088
488
§

FIG. 14

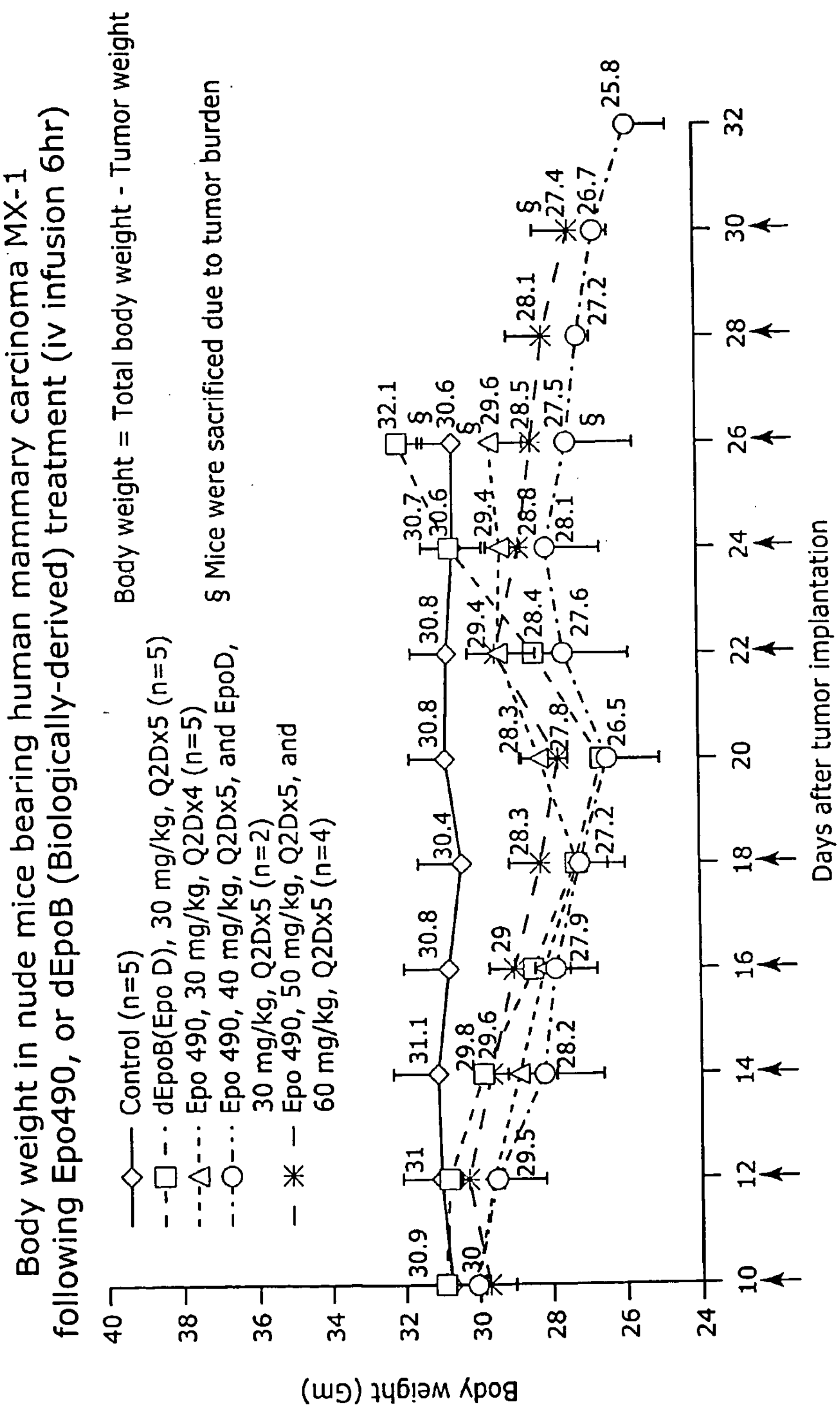
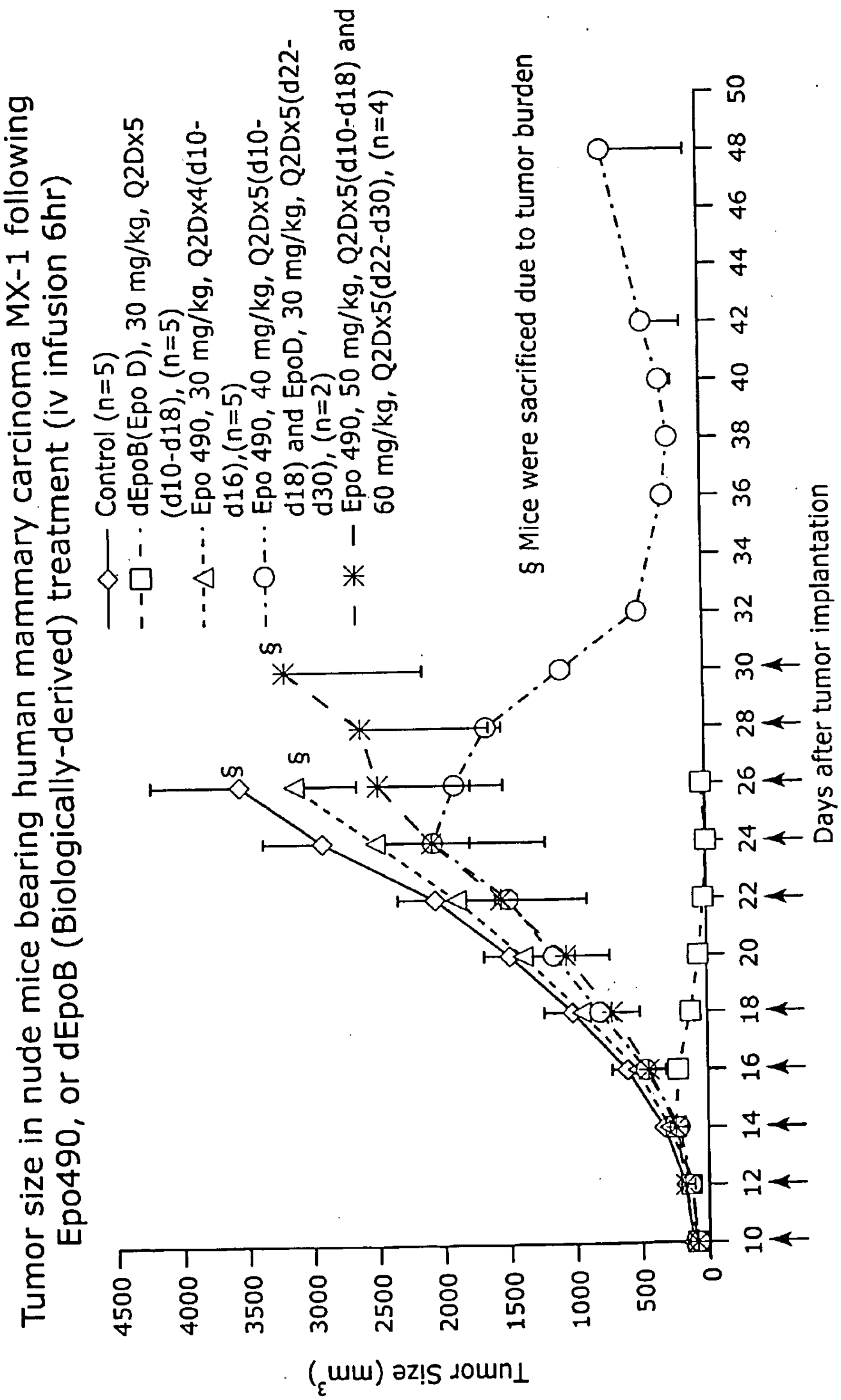

Body weight in nude mice bearing human mammary carcinoma MX-1 following Epo490, or dEpoB (Biologically-derived) treatment (iv infusion 6hr)
Control (n=5)
dEpoB(Epo D), 30 mg/kg, Q2Dx5 (n=5)
Epo 490, 30 mg/kg, Q2Dx4 (n=5)
Epo 490, 40 mg/kg, Q2Dx5, and EpoD, 30 mg/kg, Q2Dx5 (n=2)
Epo 490, 50 mg/kg, Q2Dx5, and 60 mg/kg, Q2Dx5 (n=4)
Body weight = Total body weight - Tumor weight
§ Mice were sacrificed due to tumor burden
Body weight (Gm)
Days after tumor implantation

FIG. 15



Tumor size in nude mice bearing human mammary carcinoma MX-1 following Epo490, or dEpoB (Biologically-derived) treatment (iv infusion 6hr)
Control (n=5)
dEpoB(Epo D), 30 mg/kg, Q2Dx5 (d10-d18), (n=5)
Epo 490, 30 mg/kg, Q2Dx4(d10-d16),(n=5)
Epo 490, 40 mg/kg, Q2Dx5(d10-d18) and EpoD, 30 mg/kg, Q2Dx5(d22-d30), (n=2)
Epo 490, 50 mg/kg, Q2Dx5(d10-d18) and 60 mg/kg, Q2Dx5(d22-d30), (n=4)
§ Mice were sacrificed due to tumor burden
Tumor Size (mm³)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
Days after tumor implantation

FIG. 16

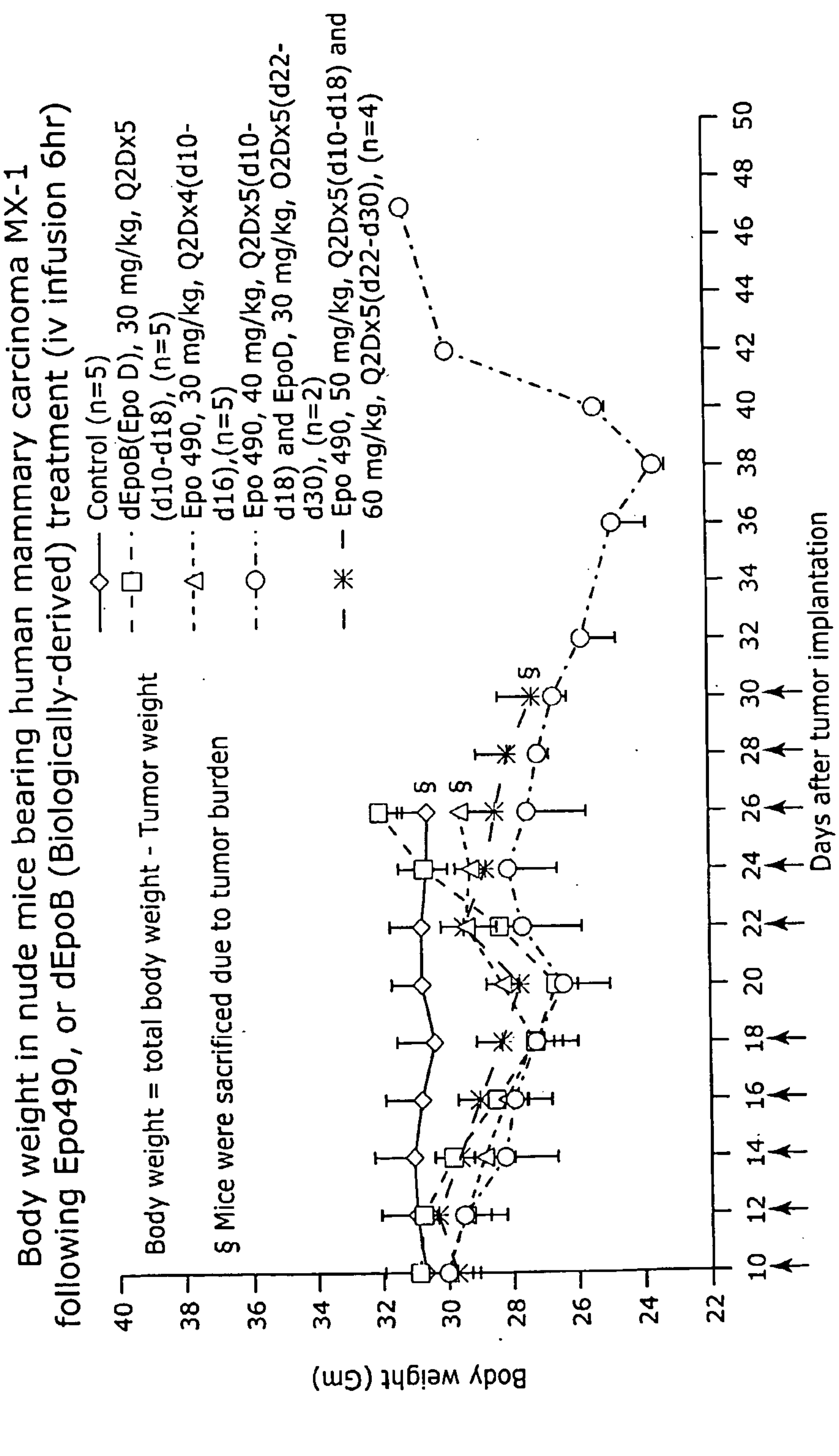

Body weight in nude mice bearing human mammary carcinoma MX-1 following Epo490, or dEpoB (Biologically-derived) treatment (iv infusion 6hr)
Body weight = total body weight - Tumor weight
§ Mice were sacrificed due to tumor burden
Control (n=5)
dEpoB(Epo D), 30 mg/kg, Q2Dx5 (d10-d18), (n=5)
Epo 490, 30 mg/kg, Q2Dx4(d10-d16),(n=5)
Epo 490, 40 mg/kg, Q2Dx5(d10-d18) and EpoD, 30 mg/kg, Q2Dx5(d22-d30), (n=2)
Epo 490, 50 mg/kg, Q2Dx5(d10-d18) and 60 mg/kg, Q2Dx5(d22-d30), (n=4)
Body weight (Gm)
40
38
36
34
32
30
28
26
24
22
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
§
§
§
Days after tumor implantation

FIG. 17

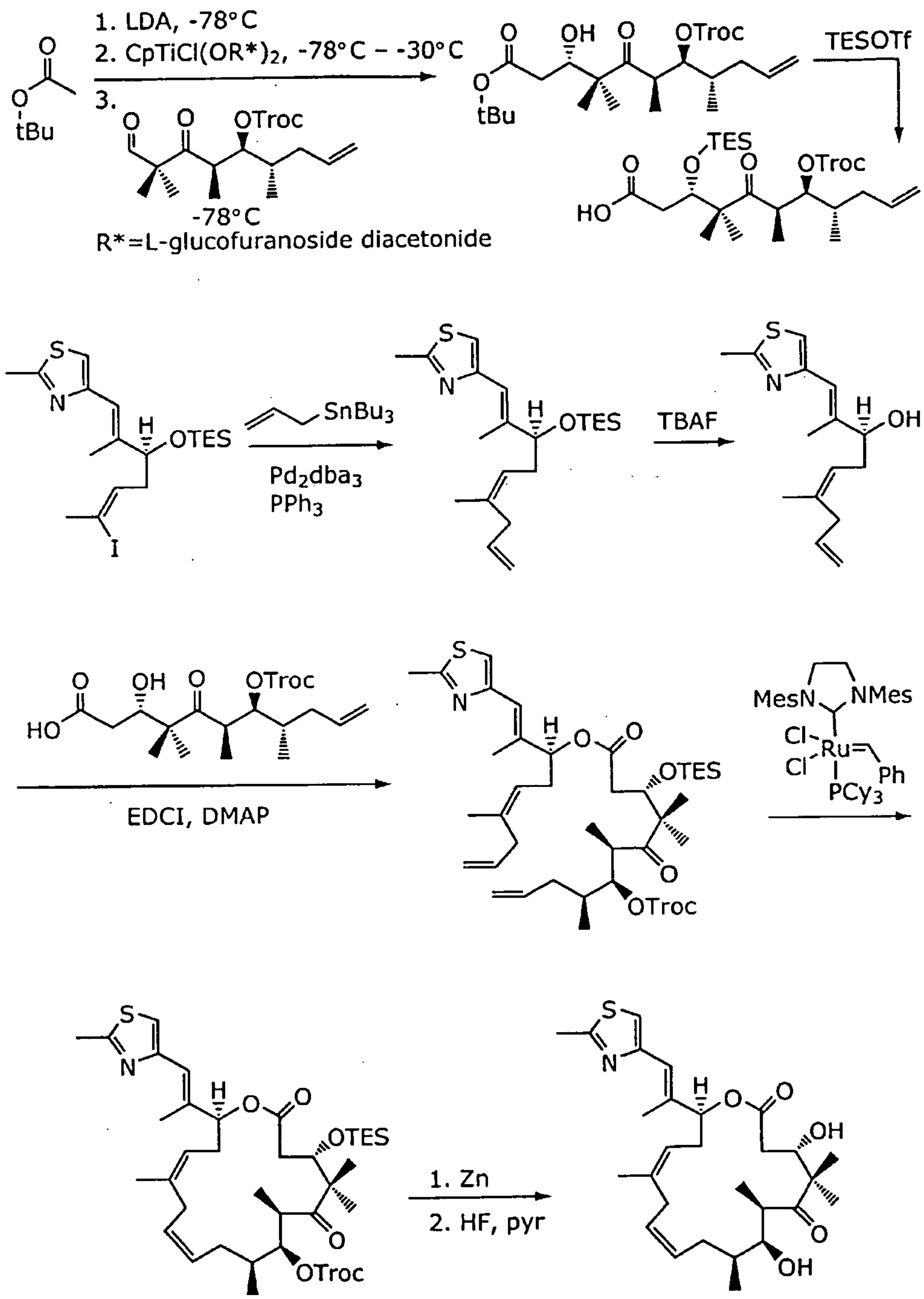

1. LDA, -78°C
2. CpTiCl(OR*)2, -78°C - -30°C
3.
-78°C
R*=L-glucofuranoside diacetonide
OTroc
TESOTf
TES
tBu
SnBu3
Pd2dba3
PPh3
OTES
TBAF
OH
EDCI, DMAP
MesN
NMes
PCy3
Ph
1. Zn
2. HF, pyr

FIG. 18

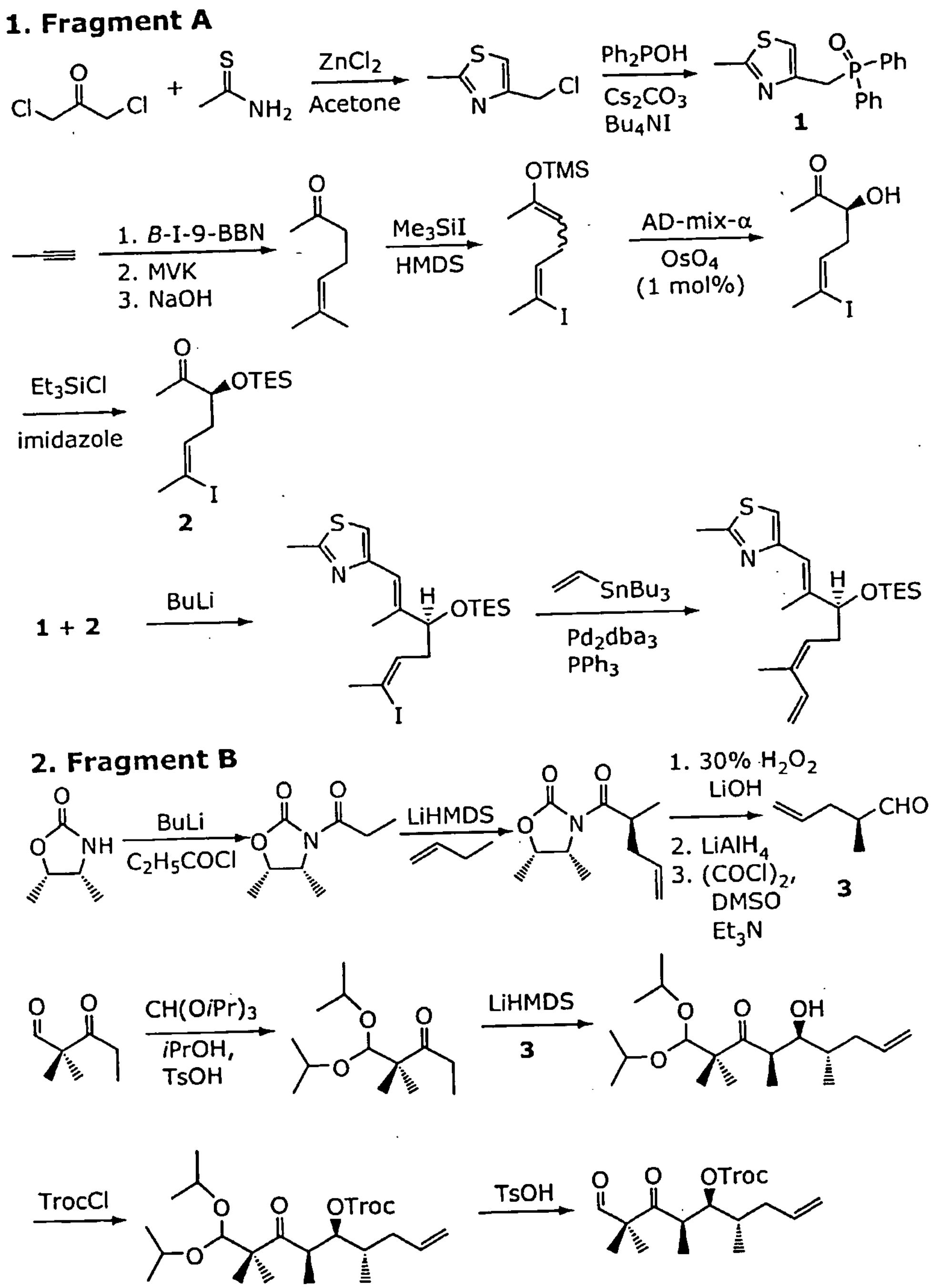

1. Fragment A
ZnCl2
Acetone
Ph2POH
Cs2CO3
Bu4NI
1
1. B-I-9-BBN
2. MVK
3. NaOH
Me3SiI
HMDS
OTMS
AD-mix-α
OsO4
(1 mol%)
OH
Et3SiCl
imidazole
OTES
2
1 + 2
BuLi
SnBu3
Pd2dba3
PPh3
2. Fragment B
BuLi
C2H5COCl
LiHMDS
1. 30% H2O2
LiOH
2. LiAlH4
3. (COCl)2,
DMSO
Et3N
CHO
3
CH(OiPr)3
iPrOH,
TsOH
LiHMDS
3
TrocCl
OTroc
TsOH

FIG. 19

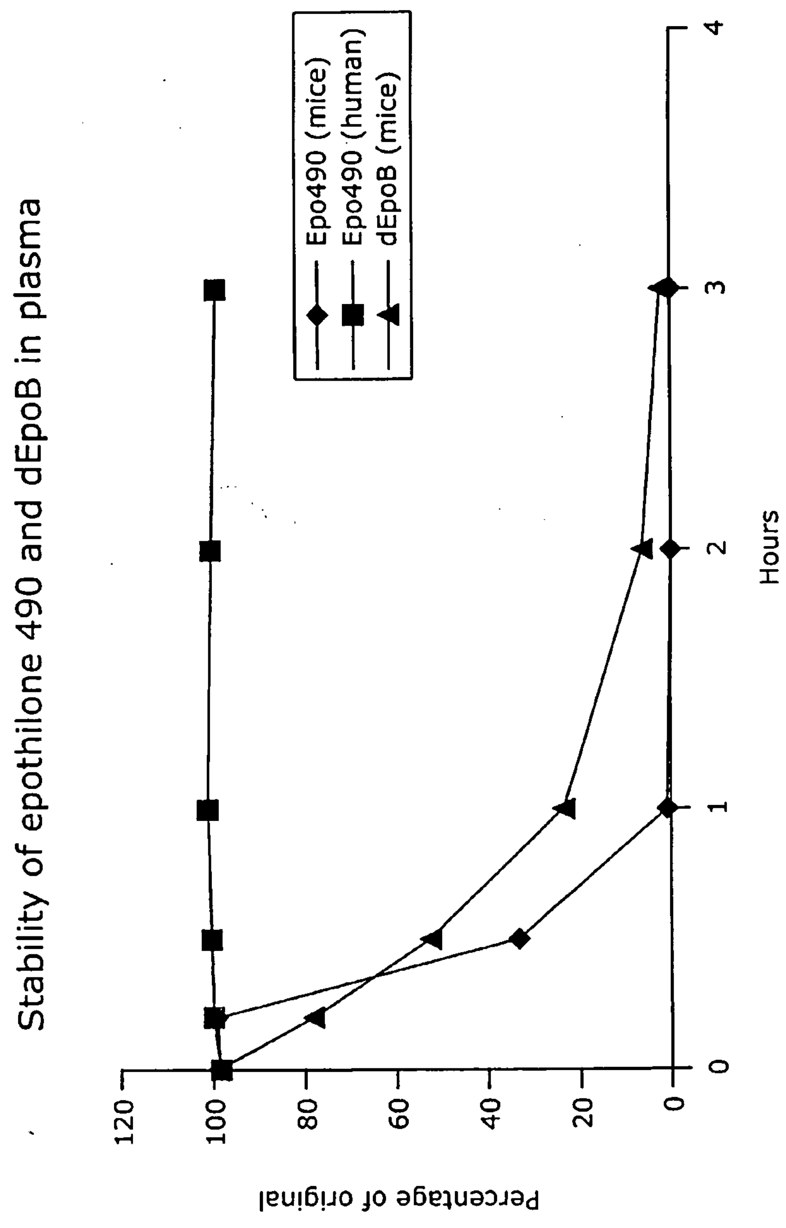

Stability of epothilone 490 and dEpoB in plasma
Epo490 (mice)
Epo490 (human)
dEpoB (mice)
Percentage of original
120
100
80
60
40
20
0
0
1
2
3
4
Hours

FIG. 21

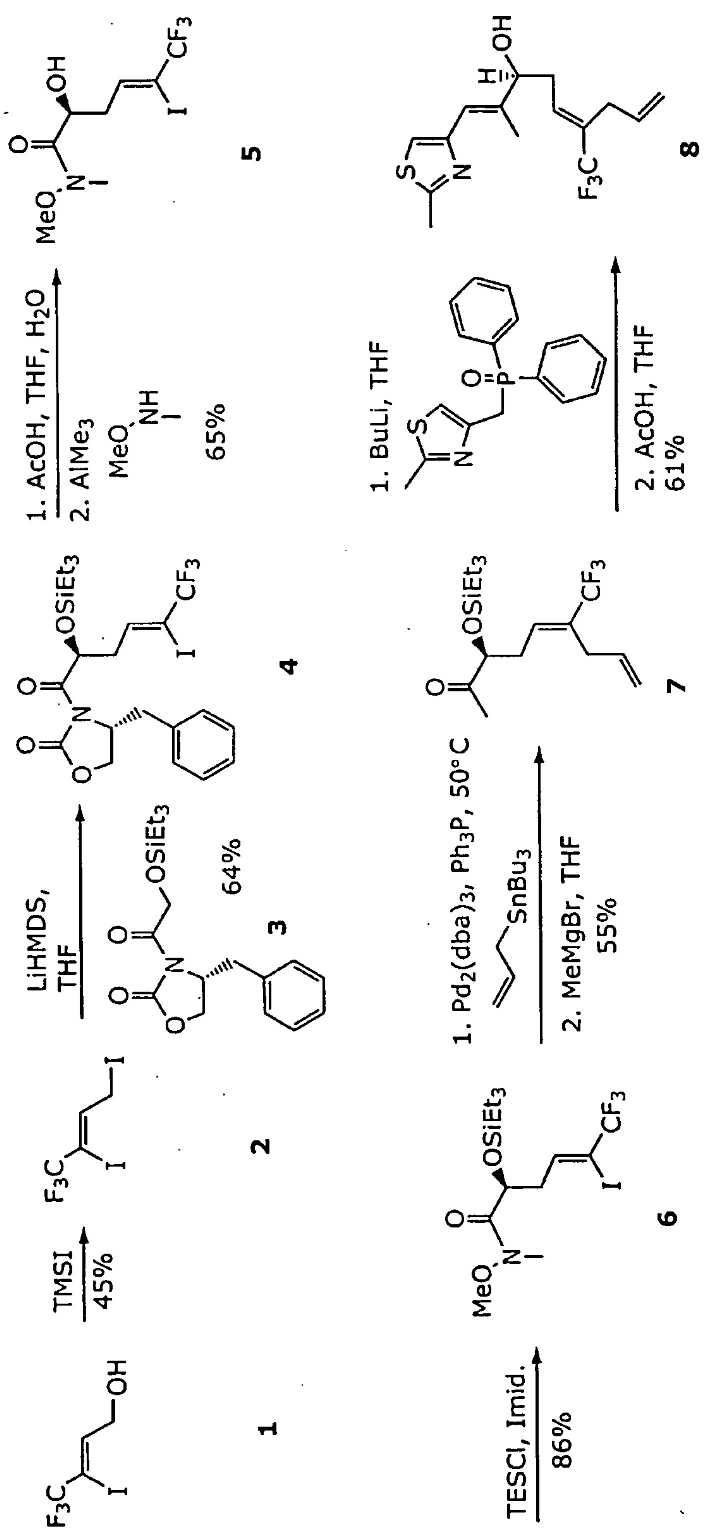


FIG. 22A

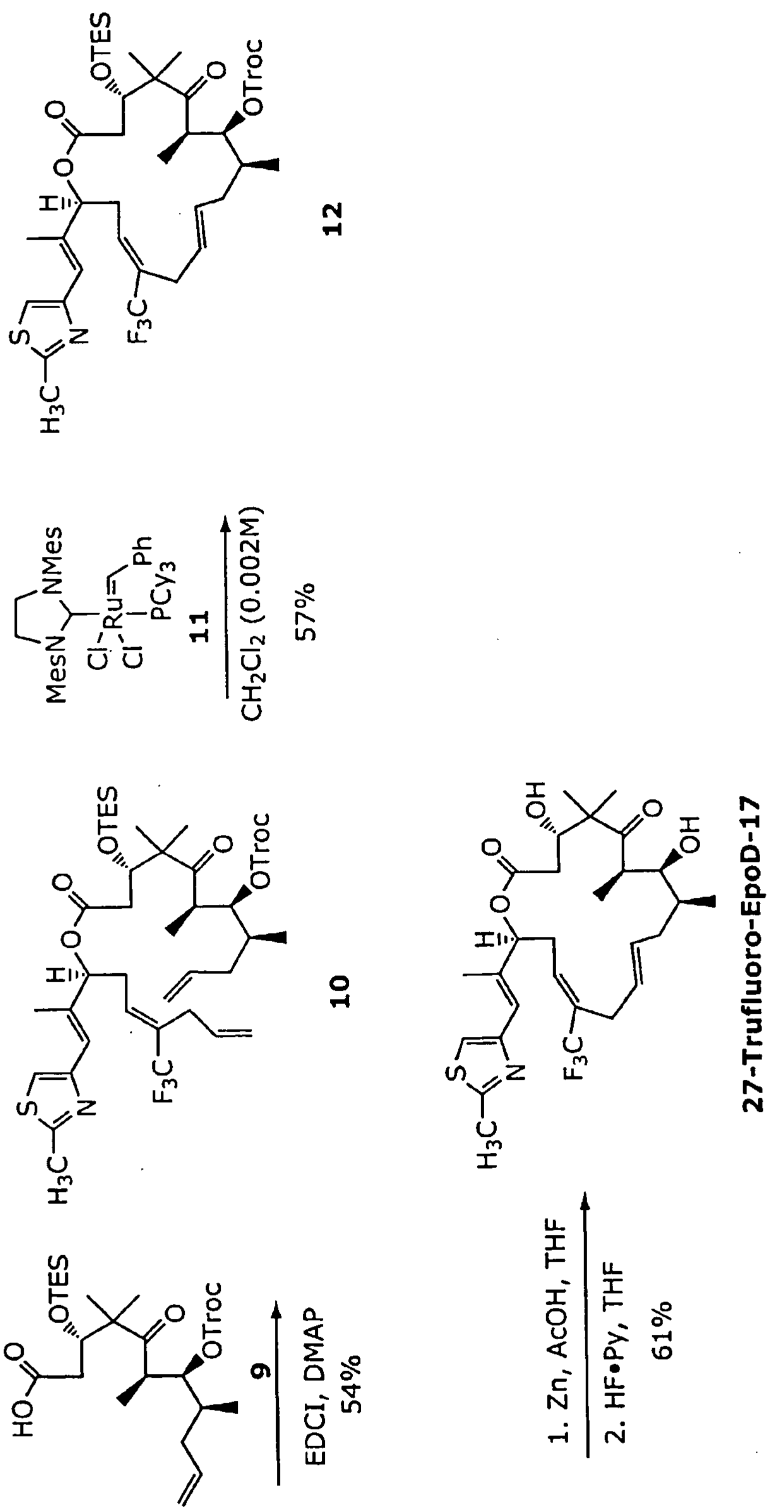

9
EDCI, DMAP
54%
10
11
CH_2Cl_2 (0.002M)
57%
12
1. Zn, AcOH, THF
2. HF•Py, THF
61%
27-Trufluoro-EpoD-17

FIG. 22B

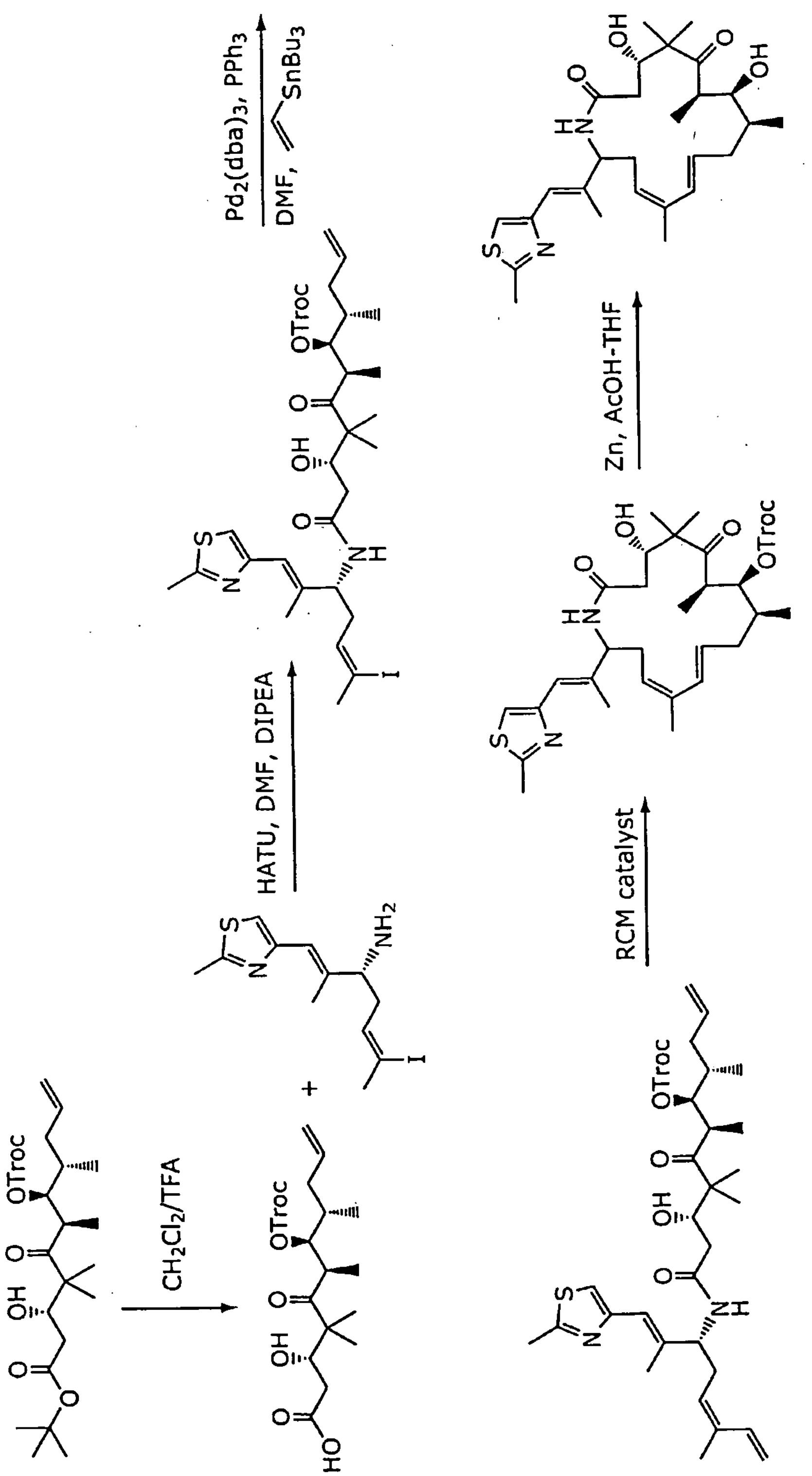

CH$_2$Cl$_2$/TFA
HATU, DMF, DIPEA
Pd$_2$(dba)$_3$, PPh$_3$
DMF, SnBu$_3$
RCM catalyst
Zn, AcOH-THF

FIG. 23

IC_{50} Comparison for CCRF-CEM Cell Lines

Compound	CCRF-CEM (μM)	CCRF-CEM/ VBL_{100} (μM)	CCRF-CEM/ VM_1 (μM)	CCRF-CEM/ Taxol (μM)
dEpoB (EpoD)	0.0047	$0.013_{[2.8x]}$	$0.016_{[2.5x]}$	$0.007_{[1.1x]}$
EpoB	0.00048	$0.0026_{[5.4x]}$	$0.0015_{[3.1x]}$	$0.0011_{[2.3x]}$
dEpoF	0.0028	$0.047_{[17.1x]}$	$0.0049_{[1.8x]}$	$0.0053_{[1.9x]}$
15-Aza-EpoB	0.0021	$2.99_{[1.423x]}$	$0.039_{[18.6x]}$	$0.171_{[81.4x]}$
Epo490 (dd-dEpoB) (10,11-didehydro EpoD)	0.020	$0.068_{[3.4x]}$	$0.035_{[1.8x]}$	$0.032_{[1.6x]}$
10,11-didehydro-dEpoF (dd-dEpoF)	0.030	$0.202_{[6.5x]}$	$0.0617_{[1.8x]}$	$0.051_{[1.6x]}$
21-Acetoxy-dd-dEpoF	0.096	$0.245_{[2.6x]}$	$0.114_{[1.2x]}$	$0.115_{[1.2x]}$
Epo-D-17 Epo[17]-490 (not effective in vivo)	0.045	$0.134_{[3.0x]}$	$0.055_{[1.2x]}$	$0.056_{[1.2x]}$
EpoD[18]-490 (not effective in vivo)	0.322	$0.870_{[2.7x]}$		$0.508_{[3.1x]}$
26-methyl-EpoD-490	0.087	$0.125_{[1.4x]}$		$0.204_{[2.3x]}$
Cyclopropyl-EpoD-490	0.077	$0.129_{[1.7x]}$		$0.181_{[2.4x]}$
10,11-di-OH-dEpoB	1.001	$99.0_{[96.9x]}$	$2.35_{[2.4x]}$	$16.76_{[16.7x]}$
10,11-ketal-dEpoB	12.21	$25.38_{[2.1x]}$	$23.33_{[1.9x]}$	$8.87_{[0.78x]}$
11-OH(Cis)EpoD	0.0044	$0.097_{[22.6x]}$	$0.0081_{[1.8x]}$	$0.012_{[2.7x]}$
27-Tri-F-[17]EpoD-490	0.068	$0.191_{[2.8x]}$		$0.326_{[4.8x]}$
HL-3-168 (Tetrahydrofuran-containing macrocycle)	1.71	$8.76_{[5.1x]}$		$4.24_{[7.5x]}$
Taxol	0.0021	$0.827_{[39.4x]}$	$0.0003_{[0.14x]}$	$0.099_{[42x]}$
VP-16	0.445	$6.75_{[15.2x]}$	$15.35_{[34.5x]}$	$2.93_{[6.6x]}$
VBL	0.00045	$0.148_{[329x]}$	$0.0014_{[3.2x]}$	$0.018_{[a0x]}$

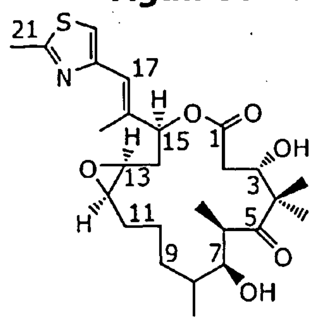
FIG. 24

IC_{50} values for the new Epothilones against CCRF-CEM cell growth

Compound	IC_{50} (μM) for		
	CCRF-CEM	CCRF-CEM/ VBL	CCRF-CEM/ Taxol
dEpoB (EpoD)	0.0036	$0.014_{[3.9x]}$	$0.0057_{[1.6x]}$
EpoB	0.00048	$0.0026_{[5.4x]}$	$0.0011_{[2.3x]}$
10,11-didehydro-dEpoB (Epo-490)	0.0160	$0.078_{[4.8]}$	$0.032_{[2x]}$
4-des-me-EpoB	0.00081	$0.0078_{[9.6x]}$	$0.017_{[2.1x]}$
11-OH (cis)EpoD	0.0029	$0.077_{[19.7x]}$	$0.0091_{[3.1x]}$
11-α-F-dEpoB	0.0285	$0.147_{[5.2x]}$	$0.0550_{[1.9x]}$
11-β-F-dEpoB	0.0980	$0.230_{[2.3x]}$	$0.138_{[1.4x]}$
19-oxazole EpoD	0.0054	$0.045_{[8.3x]}$	$0.0017_{[1.2x]}$
19-oxazole EpoB	0.00034	$0.0057_{[16.8x]}$	$0.0057_{[1.6x]}$
19-oxazole Epo490	0.0077	$0.0227_{[3.1x]}$	$0.0130_{[1.8x]}$
Taxol	0.0021	$2.30_{[2556x]}$	$0.089_{[42x]}$
Vinblastine	0.00045	$0.313_{[135x]}$	$0.018_{[40x]}$

FIG. 25

Relative Cytotoxicity of Epothilones Against Human Leukemic Cells in Vitro

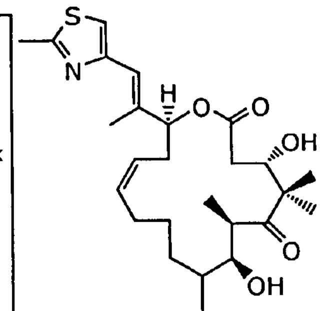


epothilone A
(0.0027)
[0.020] $_{7.4x}$

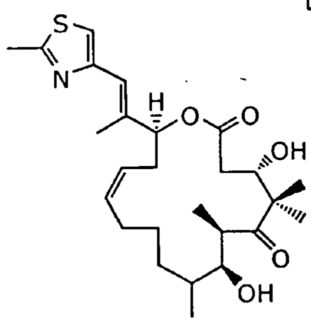
IC_{50} (μM)

VBL:	Taxol:
(0.00045)	(0.0021)
[0.182] $_{403x}$	[0.827] $_{394x}$
{0.018} $_{40x}$	{0.082} $_{39x}$

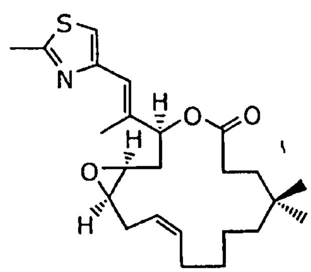
(x) = CCRF-CEM sensitive cell line
[x] = CCRF-CEM/VEL resistant cell line
{x}=CCRF-CEM/Taxol resistant cel line
Subscripts are folds of resistance.



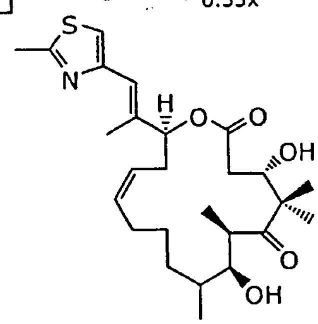
desoxyepothilone A
1
(0.022)
[0.012] $_{0.55x}$



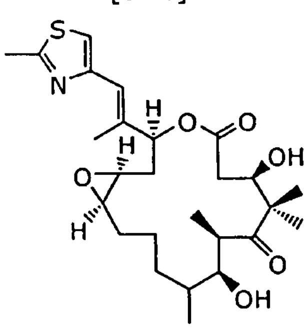
Triol-desoxyEpoA
2
(14.23)
[6.28]



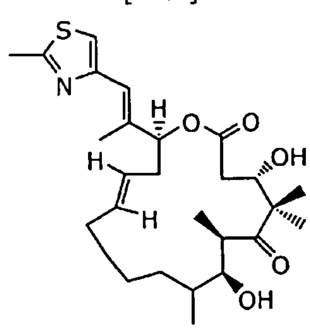
3
(271.1)
[22.4]



3-Epi-dEpoA
4
(2.12)
[43.0]

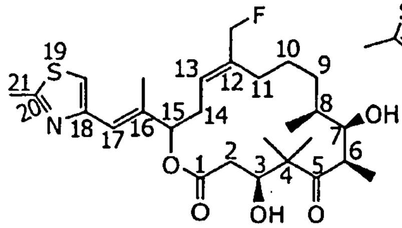


3-Epi-EpoA
5
(>20)
[35.2]

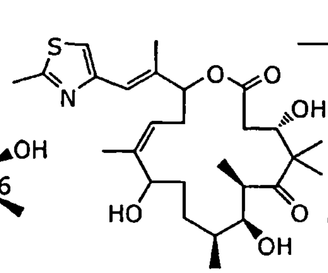


Trans-dEpoA
6
(0.052)
[0.035] $_{0.67x}$

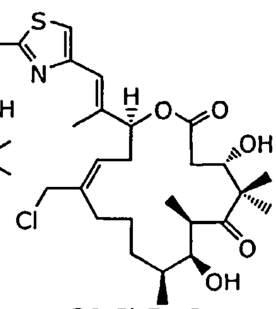
FIG. 26A



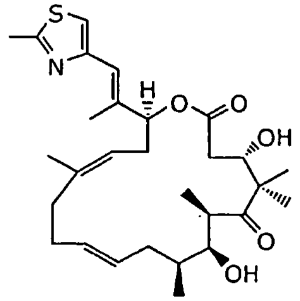
26F-Epothilone D
73
(0.040)
[0.026] 6.5x
{0.0076} 1.9x



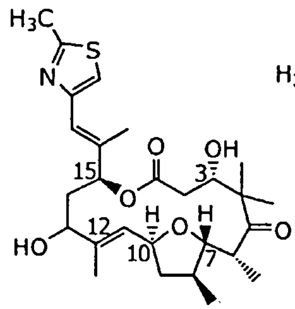
11-OH(Cis)EpoD
74
(0.0044)
[0.097] 22.6x
{0.008} 1.8x



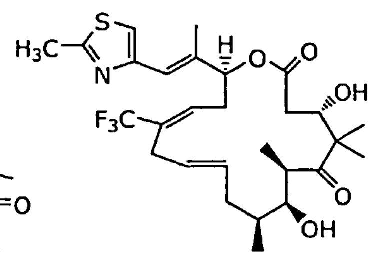
26-Cl-EpoD
75
(0.065)
[0.96] 14.5x
{0.177} 2.7x



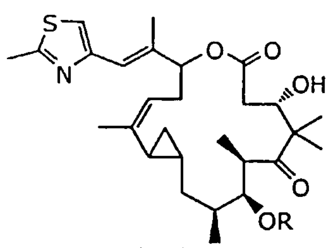
Epo-(18)-49d
76
(0.32)
[0.87] 2.7x
{0.508} 3.1x



HL-3-168
77
(1.71)
[8.8] 5.1x
{4.24} 2.5x

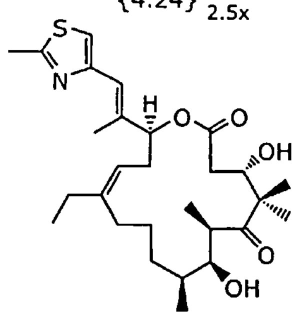


27-trifluoro-Epo-D-17-(490)
78
(0.068)
[0.191]

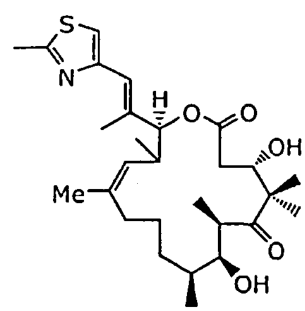


14, R = Toc
15, R = H
d

CyclopropIEpD-490
79
(0.08)
[0.13] 1.7x
{0.181} 2.4x

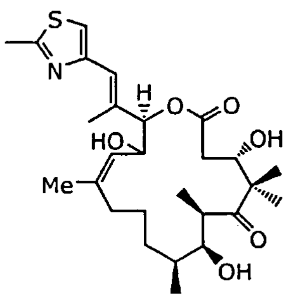


ETHYL-EPO490
80
(0.087)
[0.125] 1.4x
{0.204} 2.3x

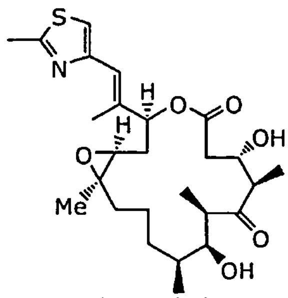


14-methylEpoD
81
(0.019)
[0.035] 1.9x
{0.022} 1.2x

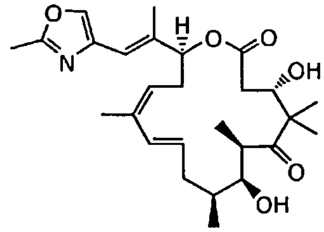
FIG. 26B



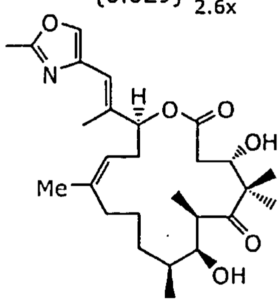
14-hydroxy-EpoD
82
(0.011)
[0.258] 23.5x
{0.029} 2.6x



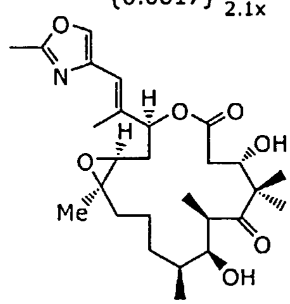
4-desmethyl-EpoB
83
(0.00081)
[0.0078] 9.6x
{0.0017} 2.1x



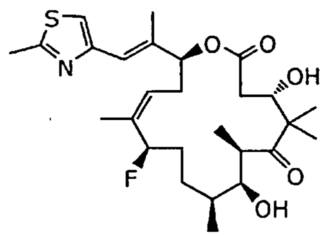
19-oxa epothilone 490
84
(0.0077)
[0.0227] 3.15x
{0.0130} 1.8x



19-oxazole-EpoD
85
(0.0054)
[0.045] 8.3x
{0.0087} 1.6x

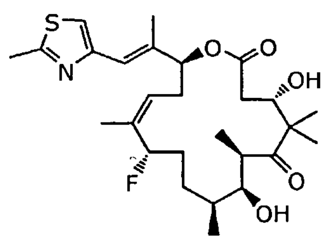


19-oxazole-EpoB
86
(0.00034)
[0.0057] 16.8x
{0.0005} 1.5x



11-α-F-dEpoB

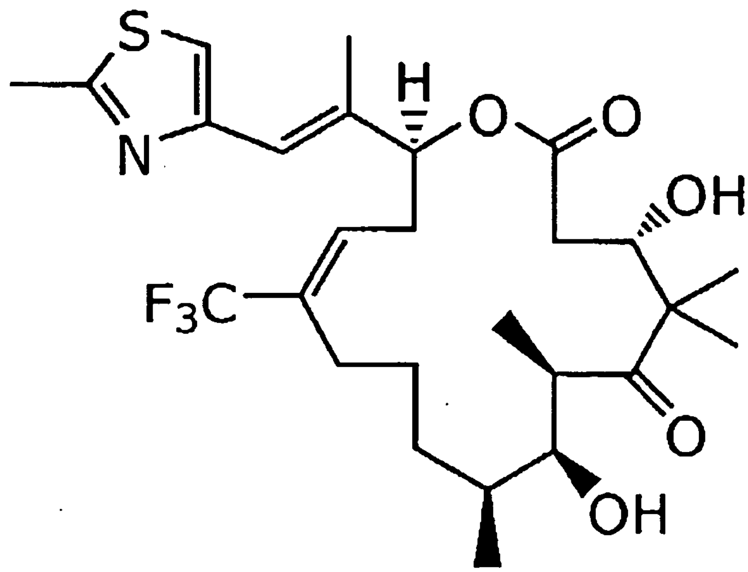
87
(0.0285)
[0.147] 5.2x
{0.0550} 1.9x



11-β-F-dEpoB

89
(0.0980)
[0.230] 2.3x
{0.138} 1.4x

FIG. 26C



26-tri-F-[16]dEpoB

90

FIG. 26D

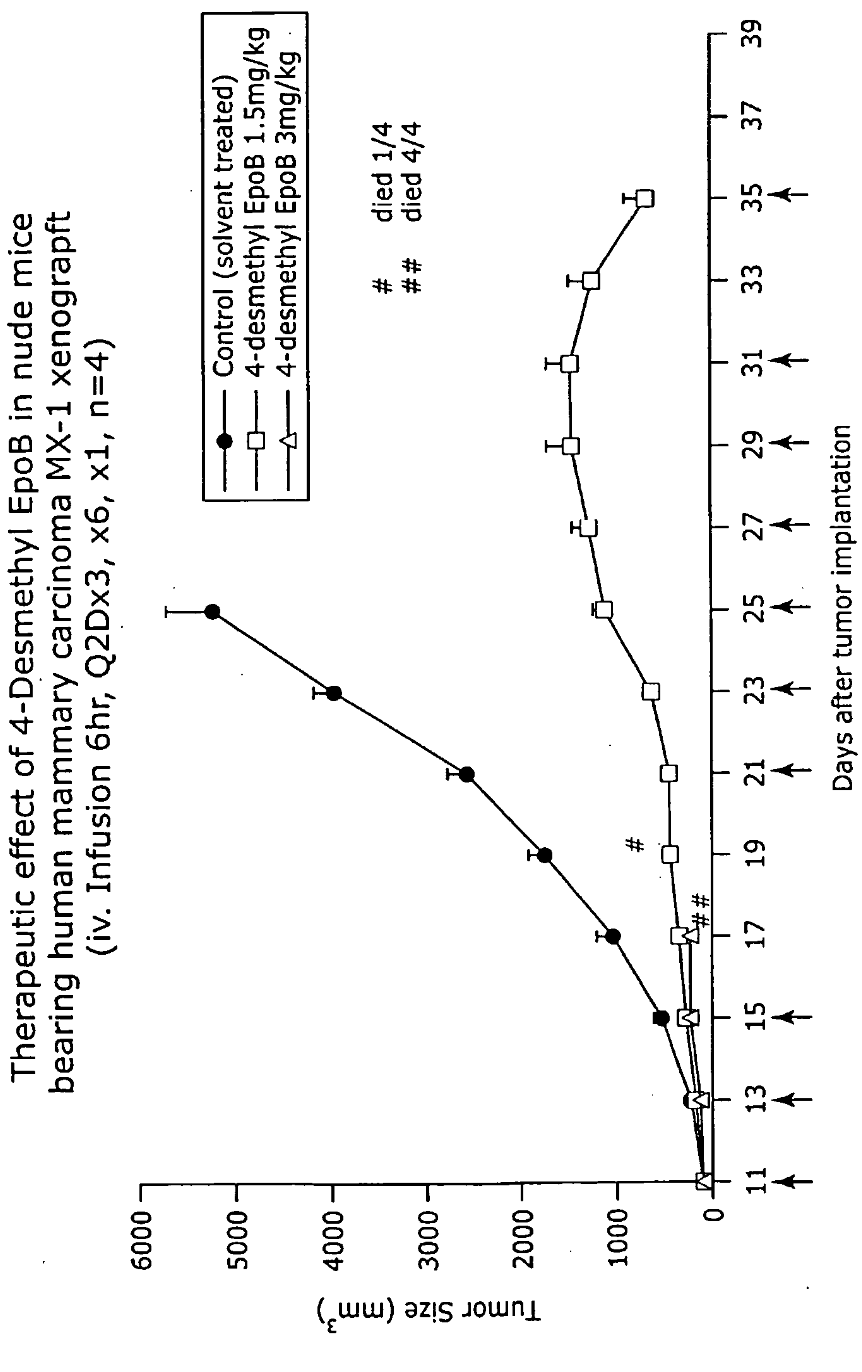

Therapeutic effect of 4-Desmethyl EpoB in nude mice
bearing human mammary carcinoma MX-1 xenograft
(iv. Infusion 6hr, Q2Dx3, x6, x1, n=4)
Control (solvent treated)
4-desmethyl EpoB 1.5mg/kg
4-desmethyl EpoB 3mg/kg
died 1/4
died 4/4
Tumor Size (mm³)
0
1000
2000
3000
4000
5000
6000
Days after tumor implantation
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39

FIG. 27

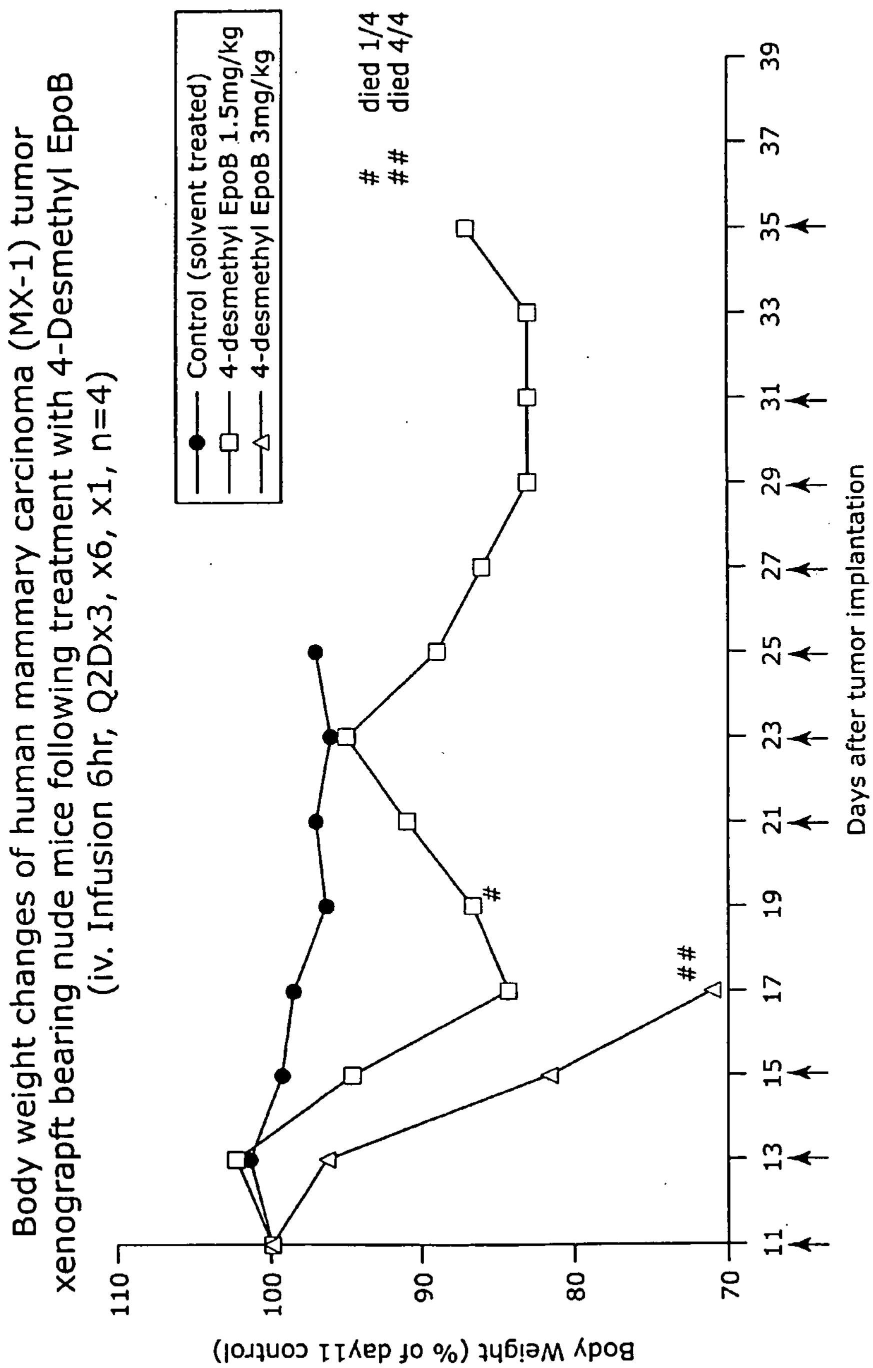

Body weight changes of human mammary carcinoma (MX-1) tumor xenograpft bearing nude mice following treatment with 4-Desmethyl EpoB
(iv. Infusion 6hr, Q2Dx3, x6, x1, n=4)
Control (solvent treated)
4-desmethyl EpoB 1.5mg/kg
4-desmethyl EpoB 3mg/kg
died 1/4
died 4/4
Body Weight (% of day11 control)
110
100
90
80
70
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
Days after tumor implantation

FIG. 28

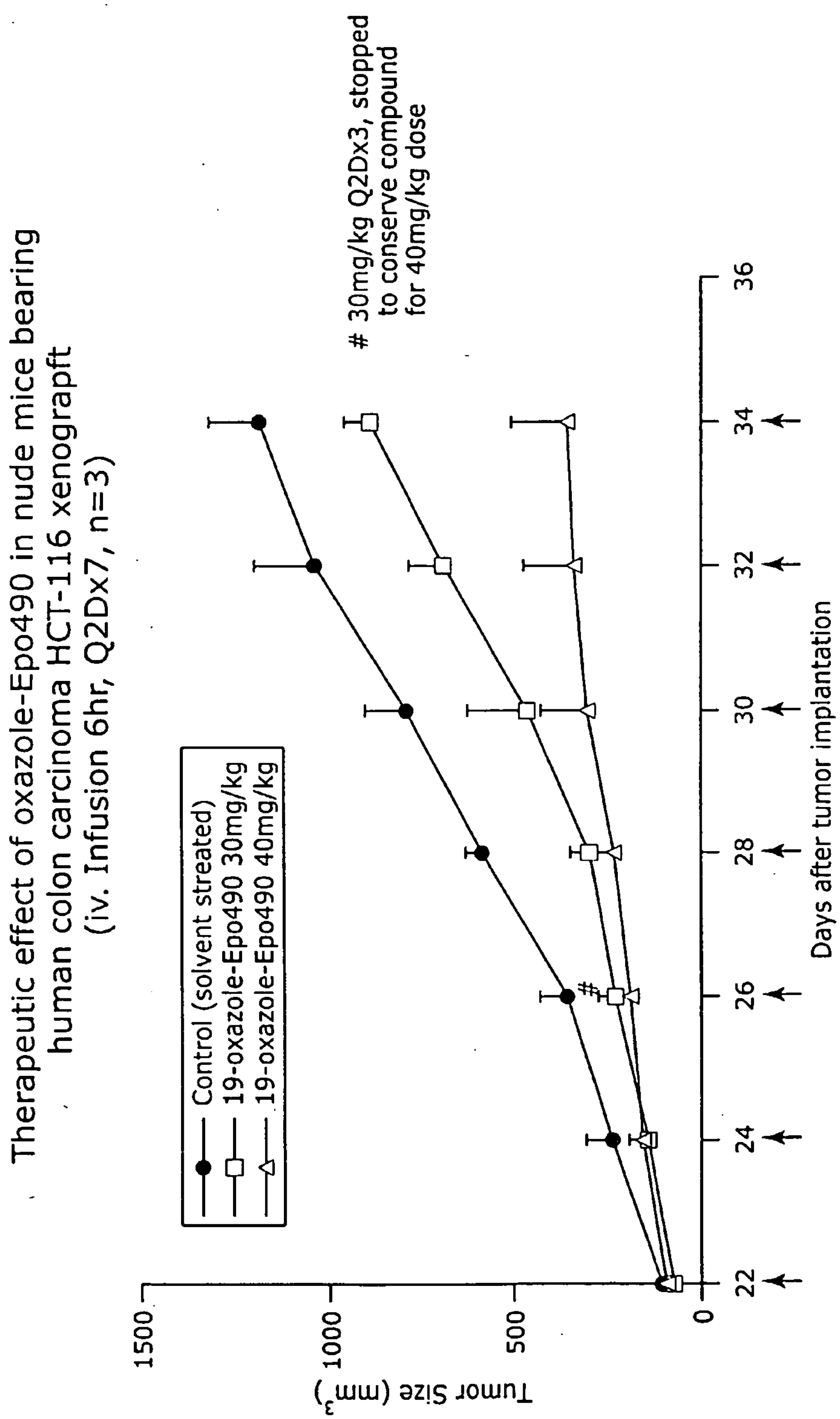

Therapeutic effect of oxazole-Epo490 in nude mice bearing
human colon carcinoma HCT-116 xenograpft
(iv. Infusion 6hr, Q2Dx7, n=3)
Control (solvent streated)
19-oxazole-Epo490 30mg/kg
19-oxazole-Epo490 40mg/kg
30mg/kg Q2Dx3, stopped
to conserve compound
for 40mg/kg dose
#
Tumor Size (mm³)
1500
1000
500
0
22
24
26
28
30
32
34
36
Days after tumor implantation

FIG. 29

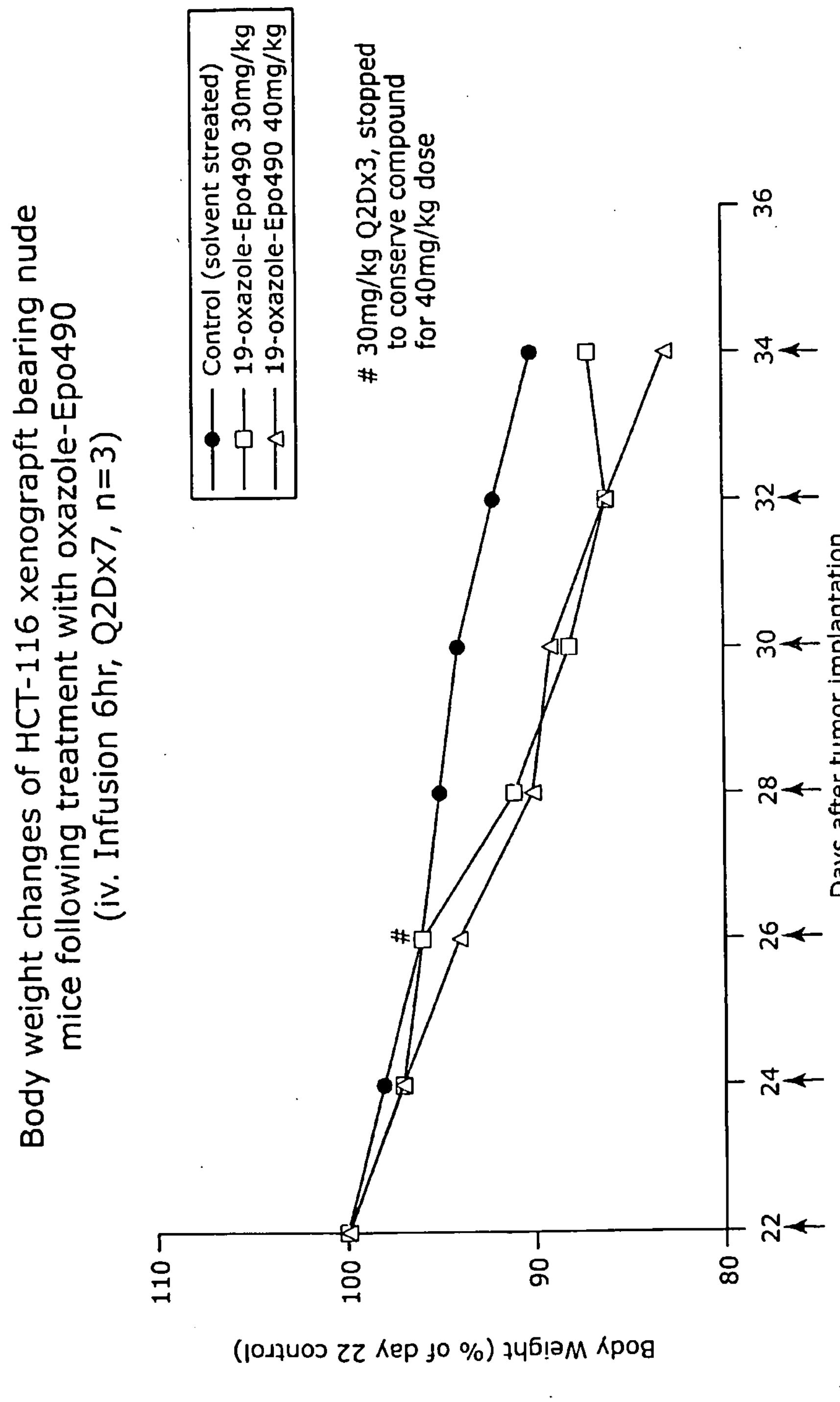

Body weight changes of HCT-116 xenograpft bearing nude mice following treatment with oxazole-Epo490
(iv. Infusion 6hr, Q2Dx7, n=3)
Control (solvent streated)
19-oxazole-Epo490 30mg/kg
19-oxazole-Epo490 40mg/kg
30mg/kg Q2Dx3, stopped to conserve compound for 40mg/kg dose
#
Body Weight (% of day 22 control)
110
100
90
80
22
24
26
28
30
32
34
36
Days after tumor implantation

FIG. 30

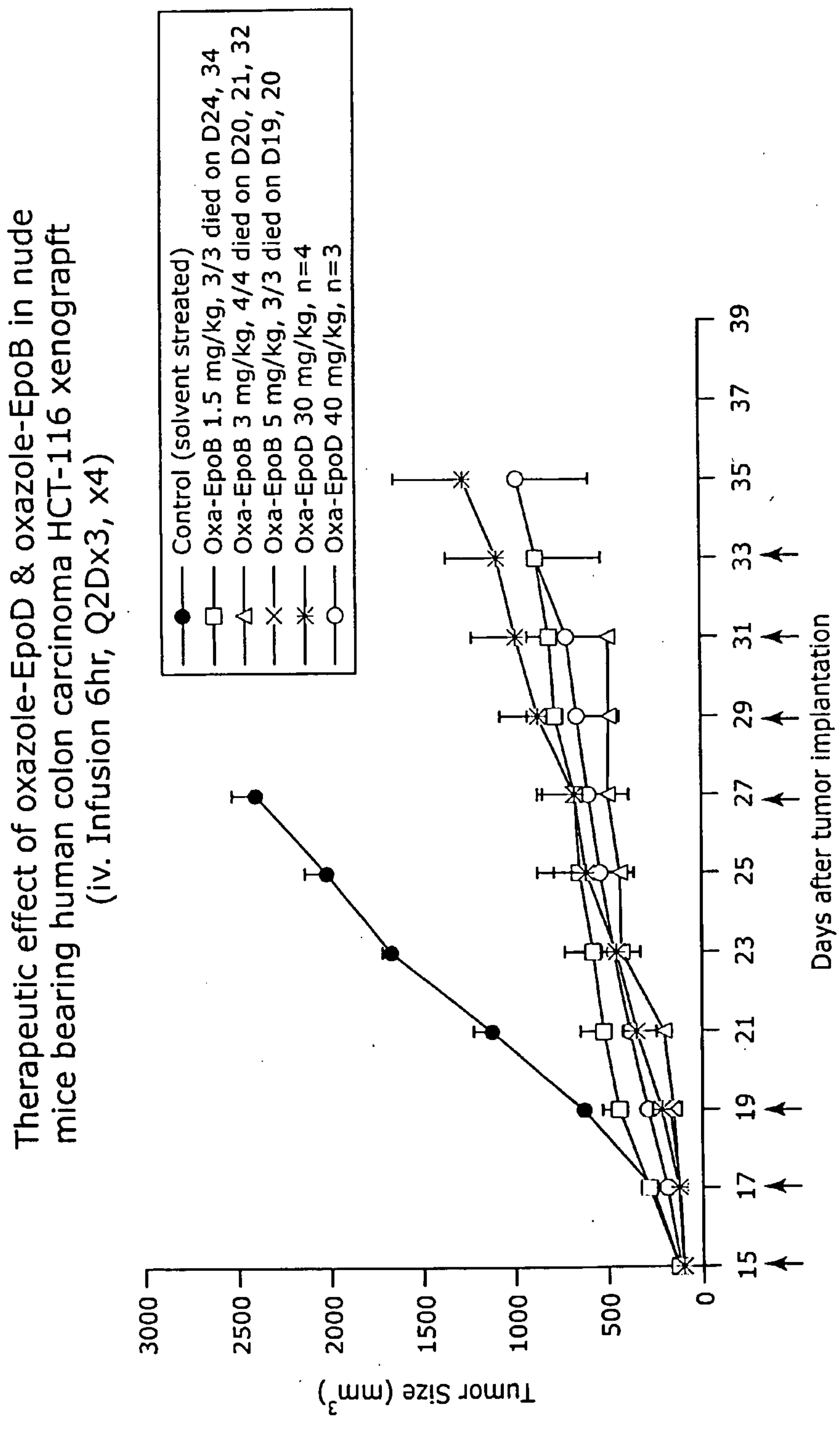

Therapeutic effect of oxazole-EpoD & oxazole-EpoB in nude mice bearing human colon carcinoma HCT-116 xenograpft
(iv. Infusion 6hr, Q2Dx3, x4)
Control (solvent streated)
Oxa-EpoB 1.5 mg/kg, 3/3 died on D24, 34
Oxa-EpoB 3 mg/kg, 4/4 died on D20, 21, 32
Oxa-EpoB 5 mg/kg, 3/3 died on D19, 20
Oxa-EpoD 30 mg/kg, n=4
Oxa-EpoD 40 mg/kg, n=3
Tumor Size (mm3)
3000
2500
2000
1500
1000
500
0
15
17
19
21
23
25
27
29
31
33
35
37
39
Days after tumor implantation

FIG. 31

Body weight changes of human colon carcinoma HCT-116 tumor xenograft bearing nude mice following treatment with oxazole-EpoD and oxazole-EpoB (iv. Infusion 6hr, Q2Dx7, n=3)

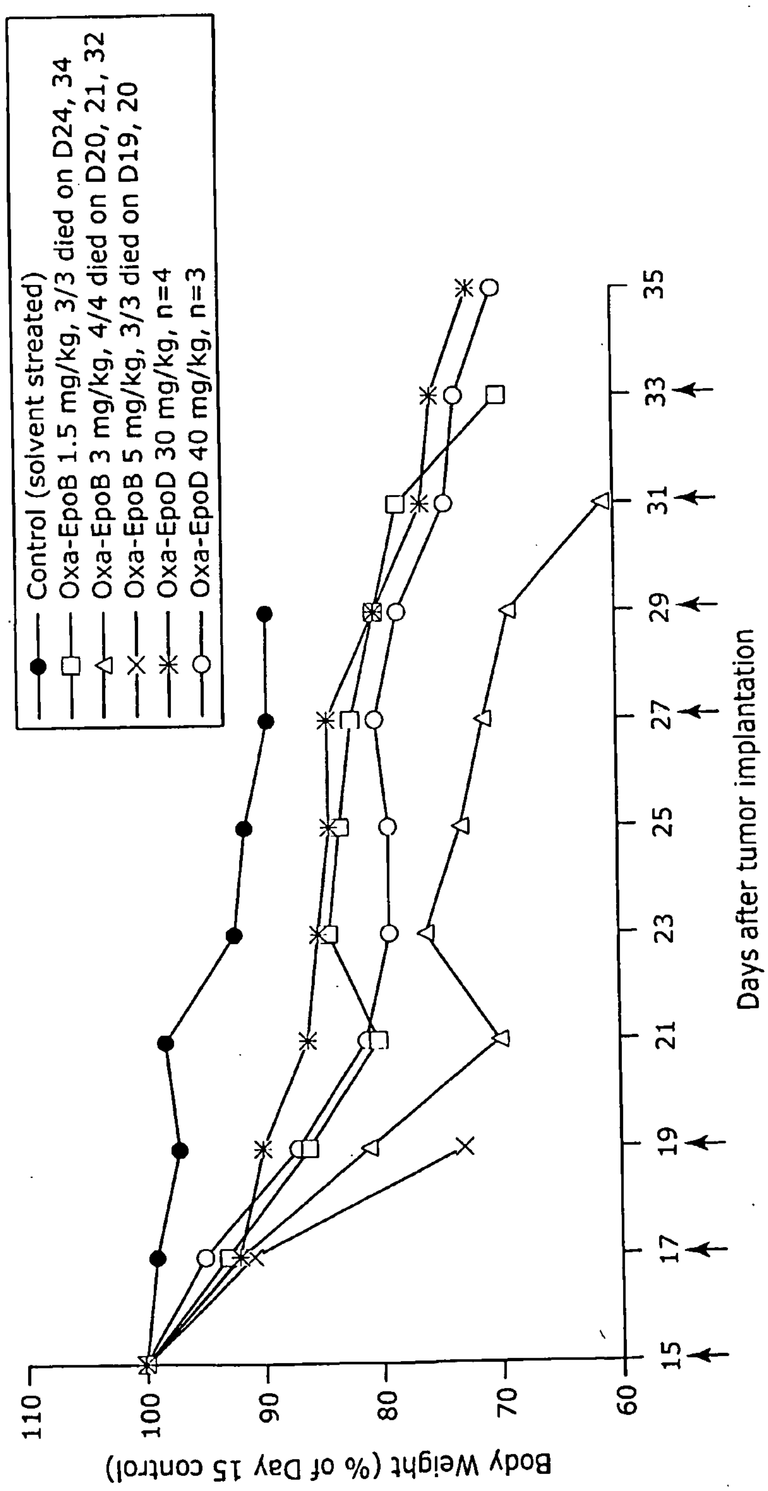


FIG. 32

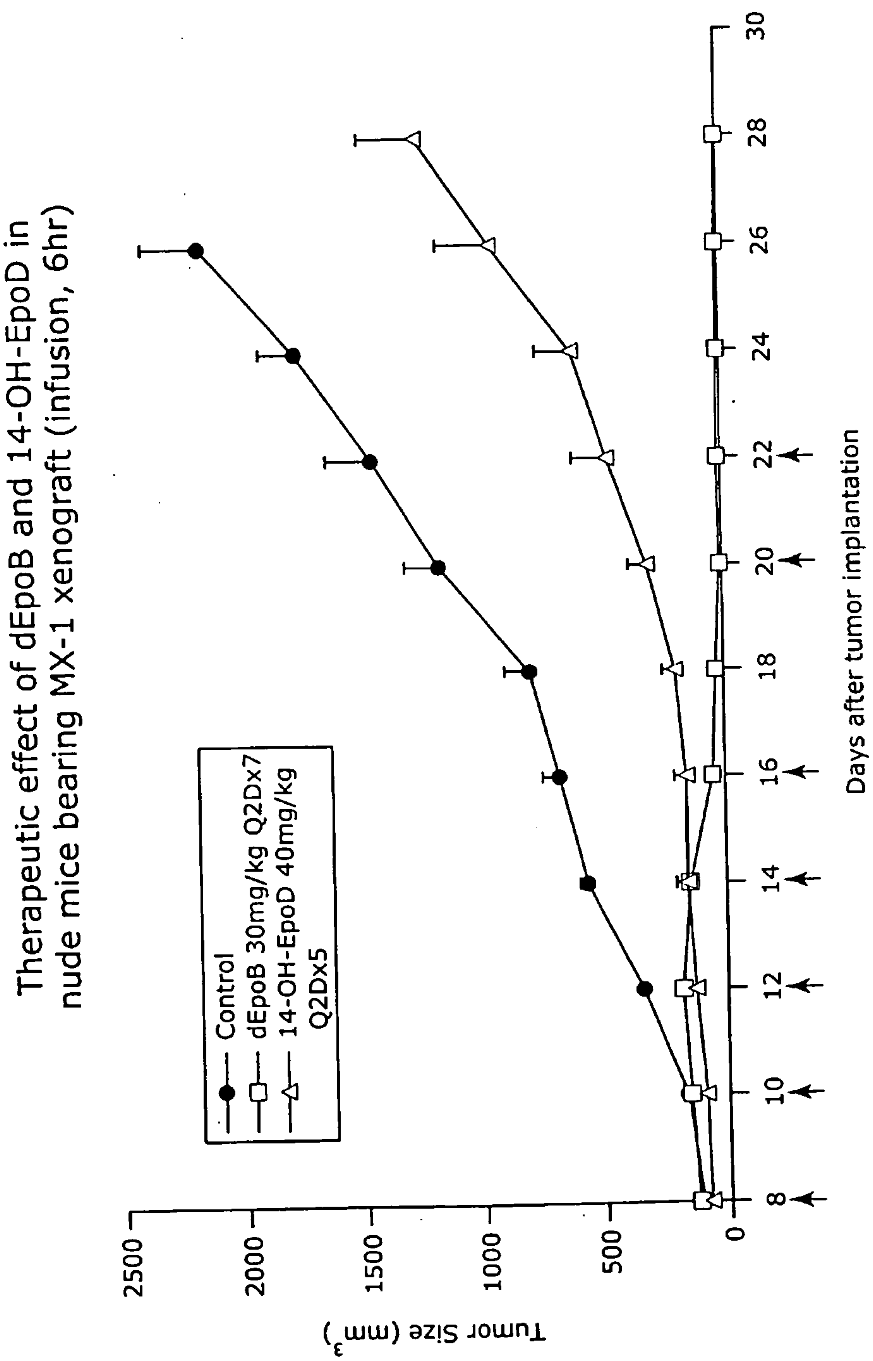

Therapeutic effect of dEpoB and 14-OH-EpoD in nude mice bearing MX-1 xenograft (infusion, 6hr)
Control
dEpoB 30mg/kg Q2Dx7
14-OH-EpoD 40mg/kg Q2Dx5
Tumor Size (mm3)
0
500
1000
1500
2000
2500
8
10
12
14
16
18
20
22
24
26
28
30
Days after tumor implantation

FIG. 33

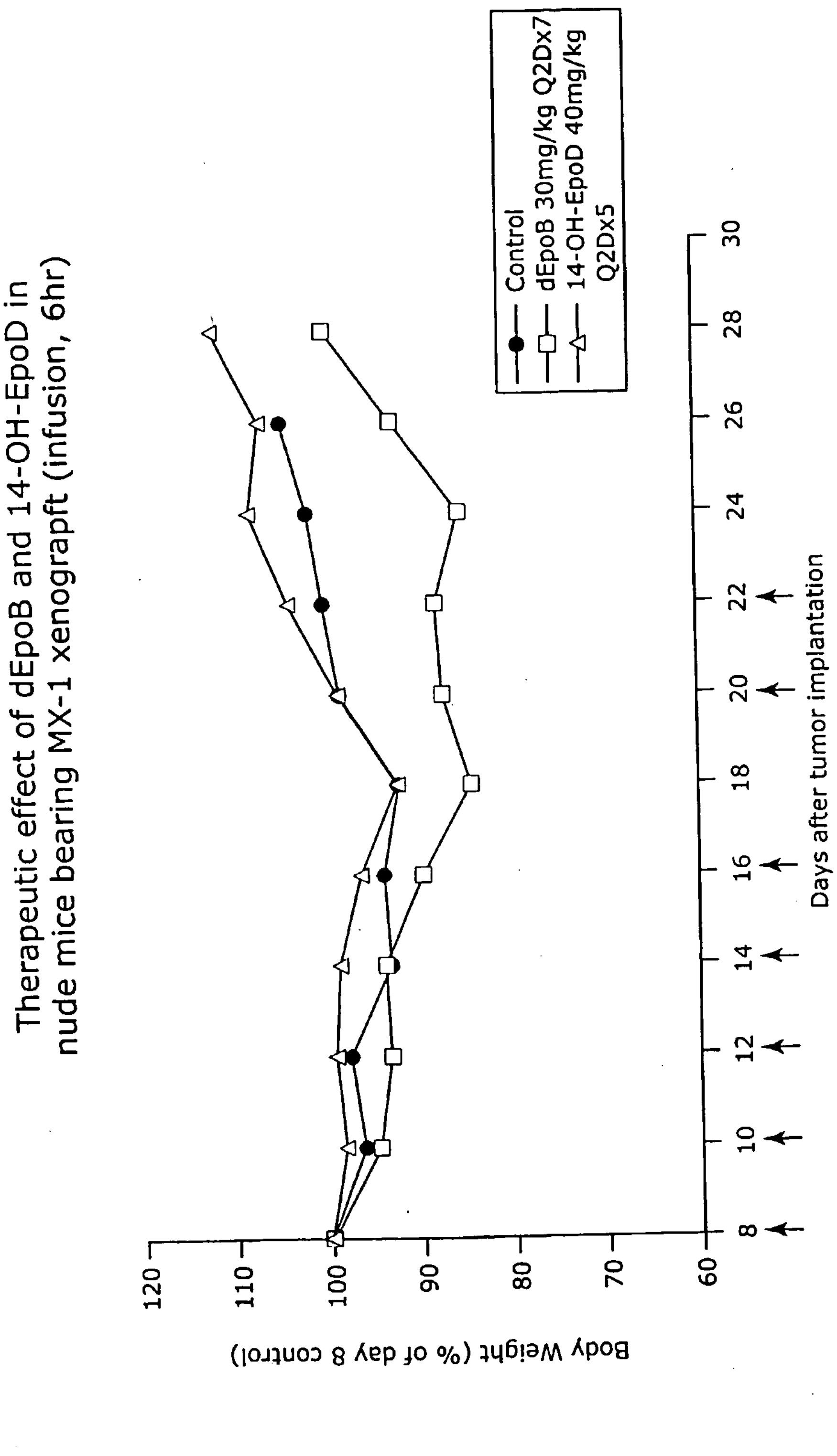

Therapeutic effect of dEpoB and 14-OH-EpoD in nude mice bearing MX-1 xenograpft (infusion, 6hr)
Control
dEpoB 30mg/kg Q2Dx7
14-OH-EpoD 40mg/kg Q2Dx5
Body Weight (% of day 8 control)
120
110
100
90
80
70
60
8
10
12
14
16
18
20
22
24
26
28
30
Days after tumor implantation

FIG. 34

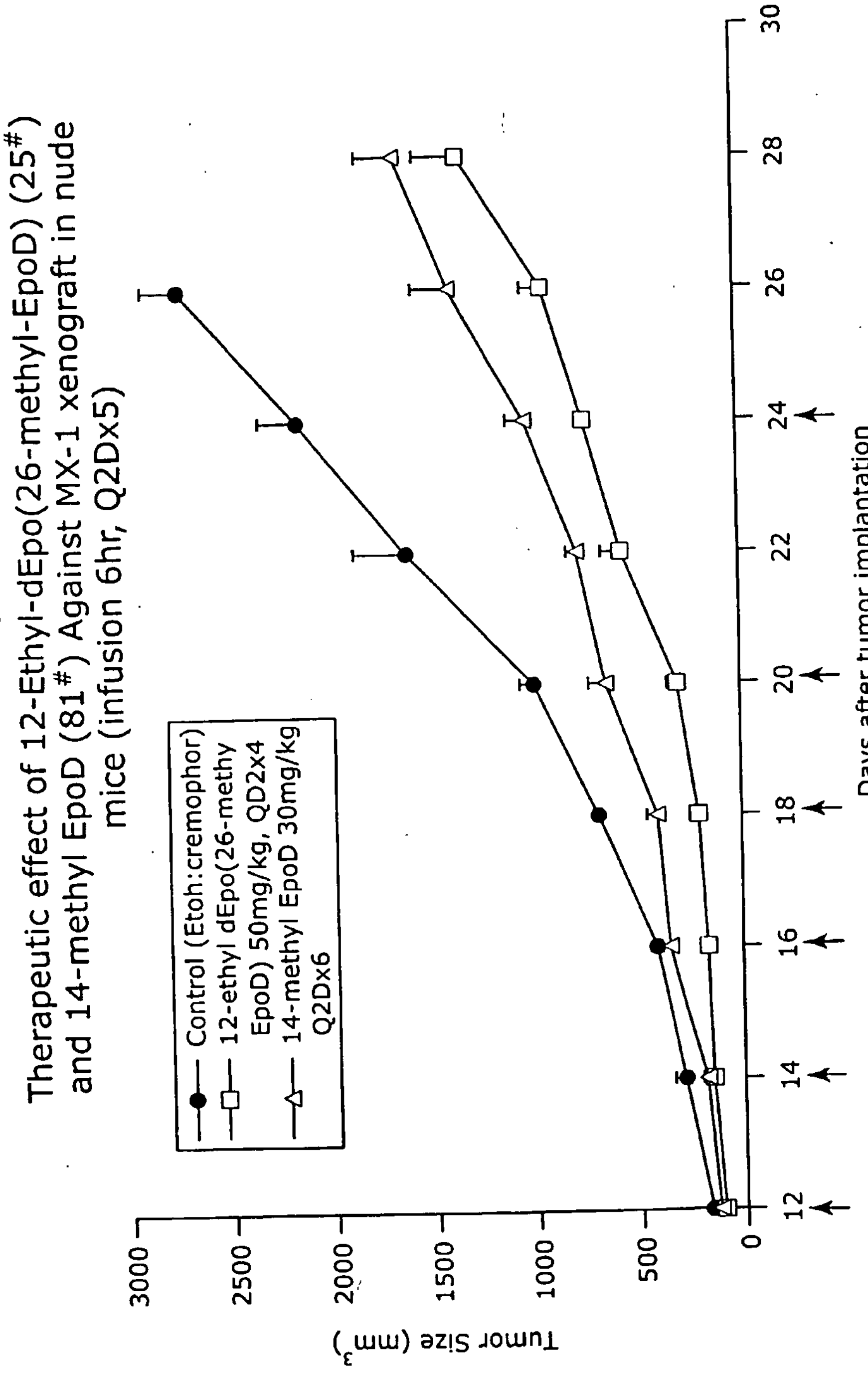

Therapeutic effect of 12-Ethyl-dEpo(26-methyl-EpoD) (25#) and 14-methyl EpoD (81#) Against MX-1 xenograft in nude mice (infusion 6hr, Q2Dx5)
Control (Etoh:cremophor)
12-ethyl dEpo(26-methy EpoD) 50mg/kg, QD2x4
14-methyl EpoD 30mg/kg Q2Dx6
Tumor Size (mm3)
0
500
1000
1500
2000
2500
3000
12
14
16
18
20
22
24
26
28
30
Days after tumor implantation

FIG. 35

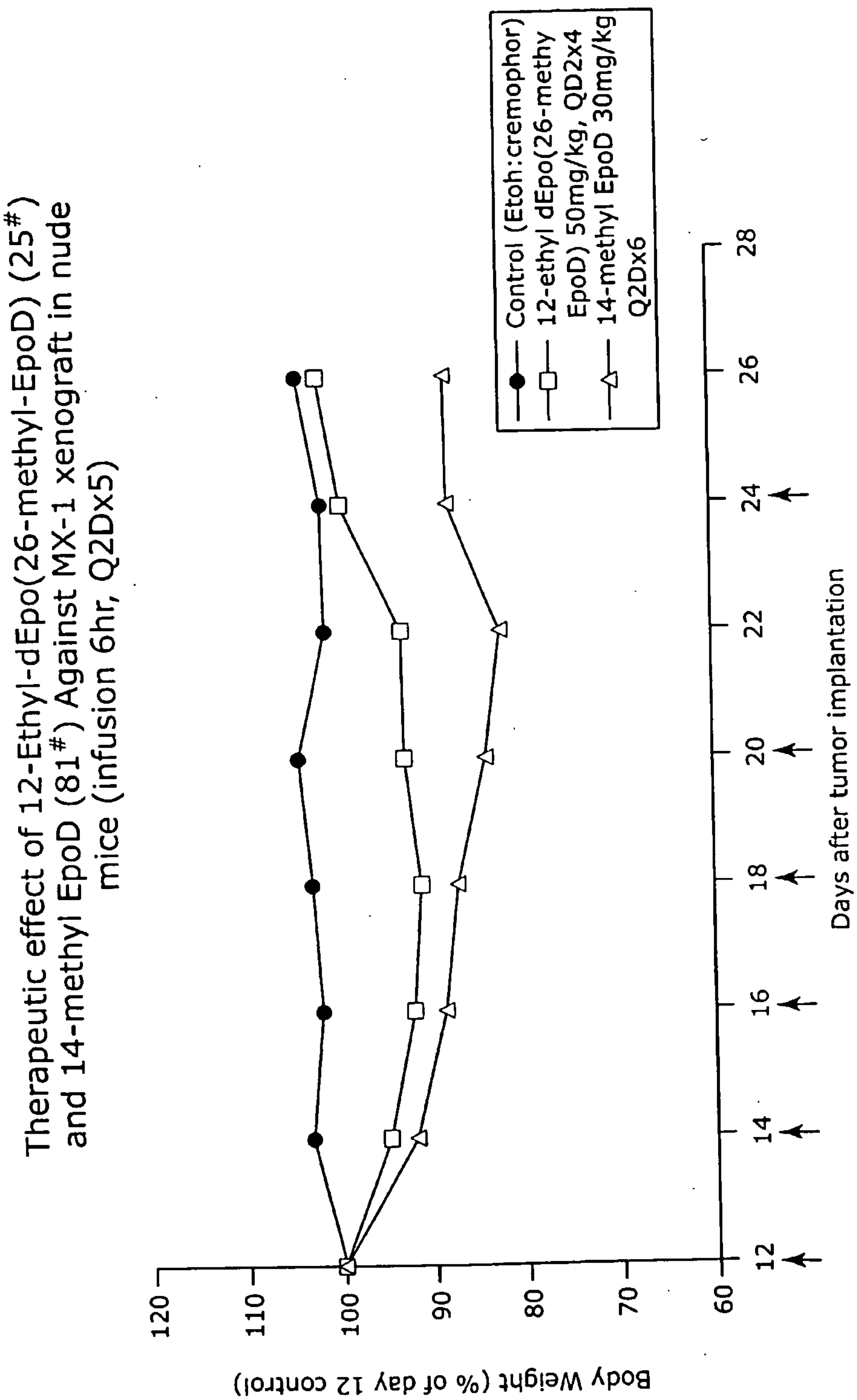

Therapeutic effect of 12-Ethyl-dEpo(26-methyl-EpoD) (25#) and 14-methyl EpoD (81#) Against MX-1 xenograft in nude mice (infusion 6hr, Q2Dx5)
Body Weight (% of day 12 control)
120
110
100
90
80
70
60
12
14
16
18
20
22
24
26
28
Days after tumor implantation
Control (Etoh:cremophor)
12-ethyl dEpo(26-methy EpoD) 50mg/kg, QD2x4
14-methyl EpoD 30mg/kg Q2Dx6

FIG. 36

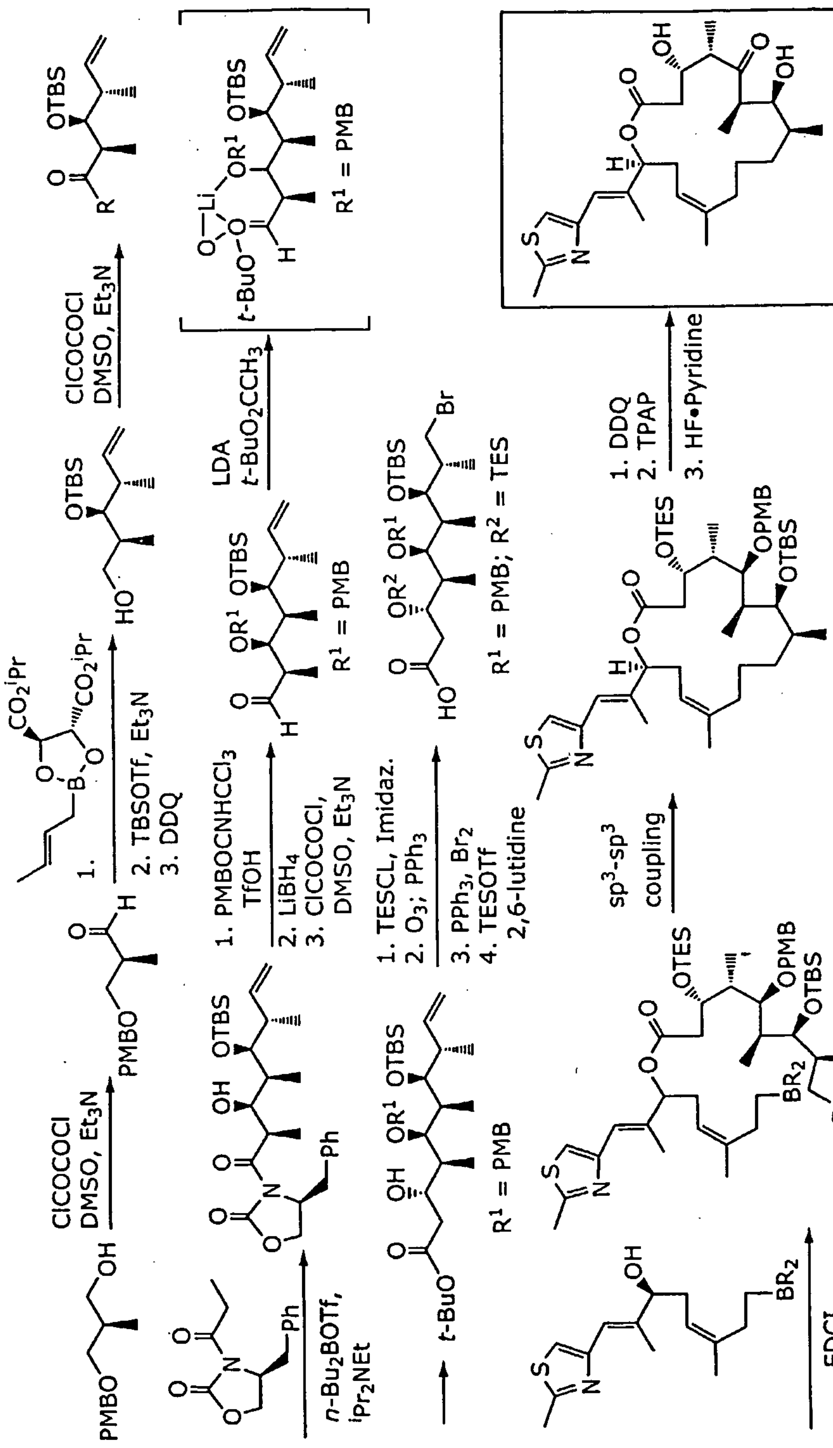

ClCOCOCl
DMSO, Et3N
1.
2. TBSOTf, Et3N
3. DDQ
ClCOCOCl
DMSO, Et3N
n-Bu2BOTf,
iPr2NEt
1. PMBOCNHCCl3
TfOH
2. LiBH4
3. ClCOCOCl,
DMSO, Et3N
R1 = PMB
LDA
t-BuO2CCH3
R1 = PMB
1. TESCL, Imidaz.
2. O3; PPh3
3. PPh3, Br2
4. TESOTf
2,6-lutidine
R1 = PMB
R1 = PMB; R2 = TES
EDCI
DMAP
sp3-sp3
coupling
1. DDQ
2. TPAP
3. HF•Pyridine

FIG. 37A

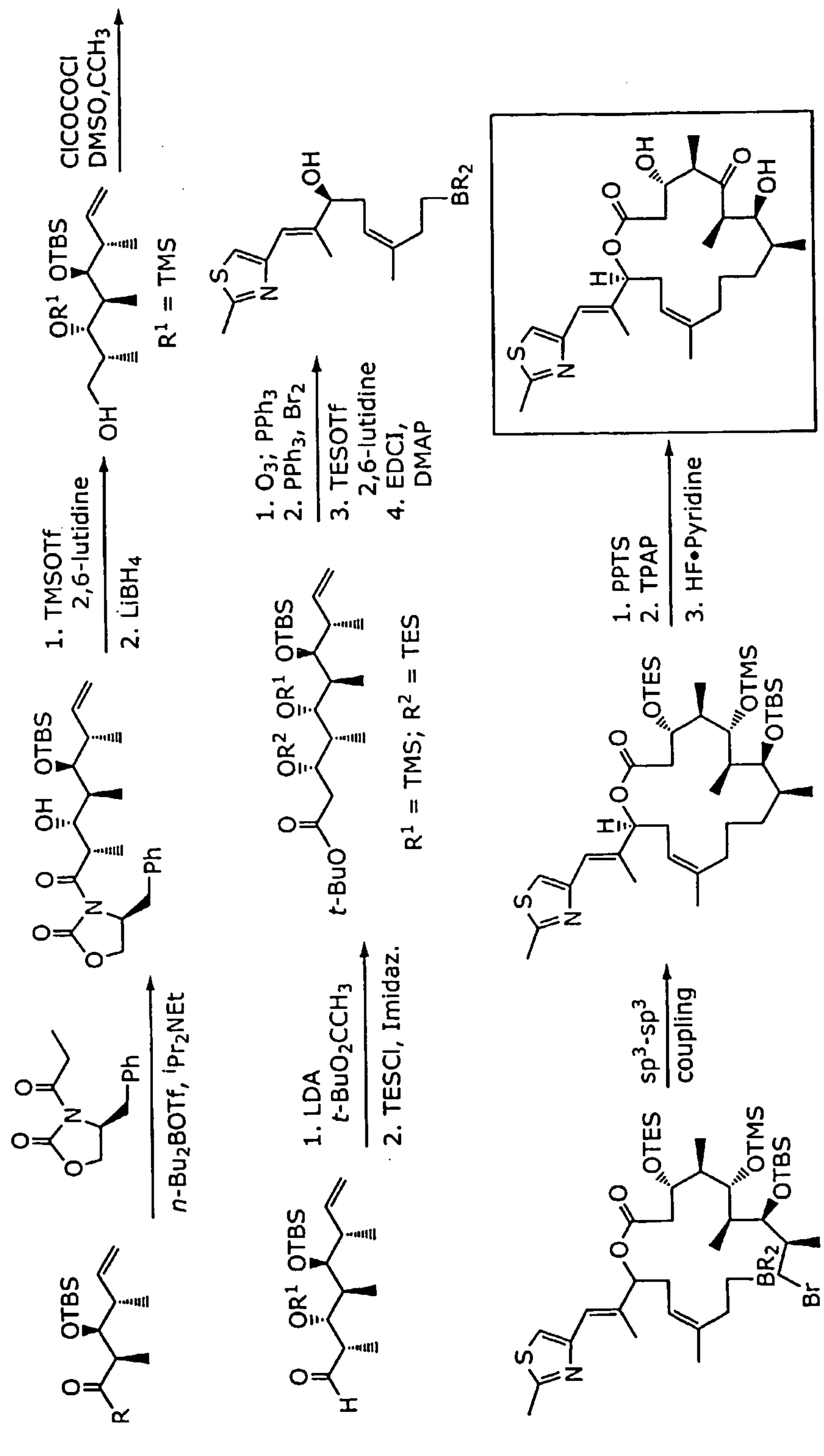


FIG. 37B

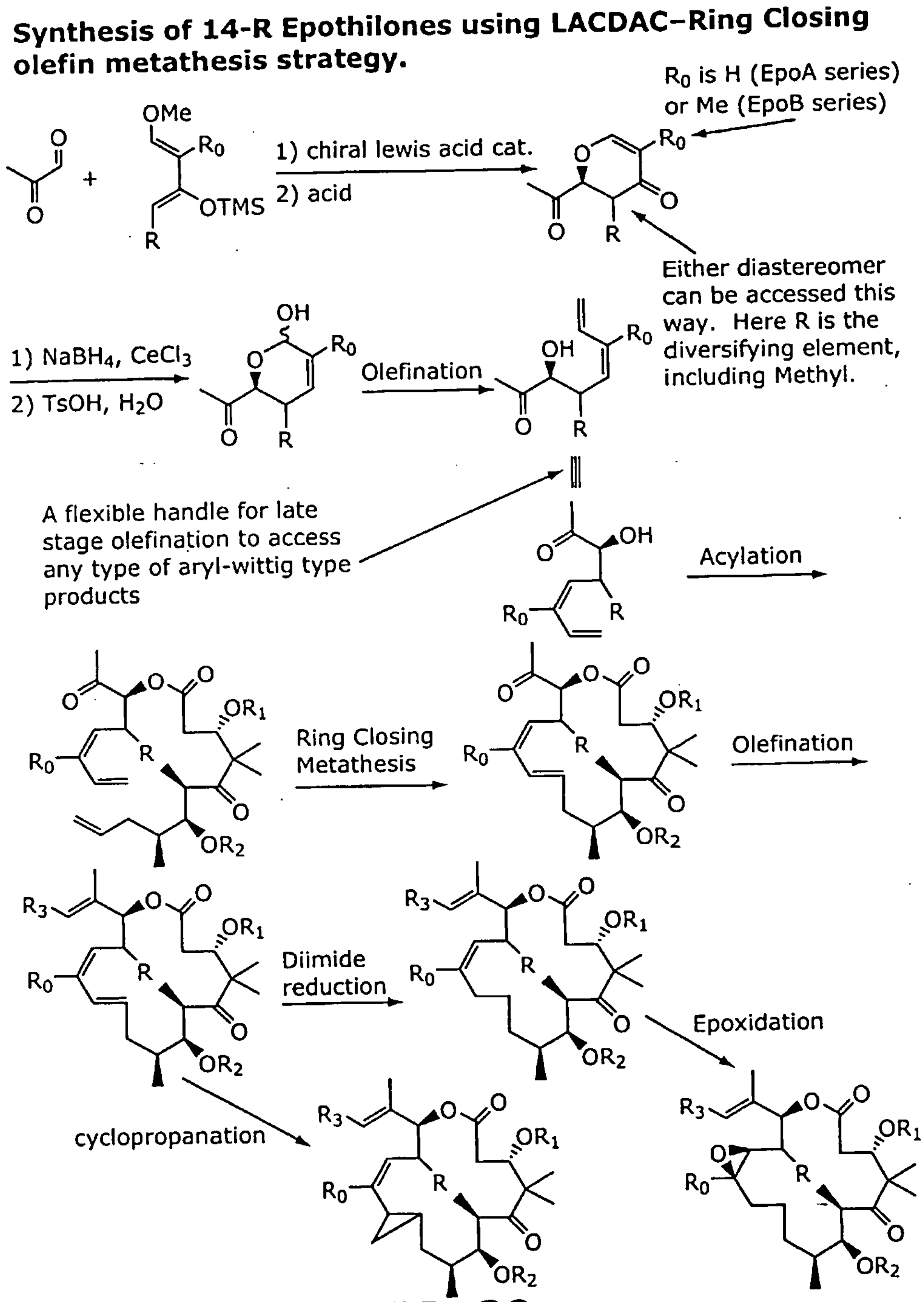

Synthesis of 14-R Epothilones using LACDAC-Ring Closing olefin metathesis strategy.
OMe
R0
OTMS
R
1) chiral lewis acid cat.
2) acid
R0 is H (EpoA series) or Me (EpoB series)
Either diastereomer can be accessed this way. Here R is the diversifying element, including Methyl.
1) NaBH4, CeCl3
2) TsOH, H2O
OH
Olefination
A flexible handle for late stage olefination to access any type of aryl-wittig type products
Acylation
OR1
OR2
Ring Closing Metathesis
Olefination
R3
Diimide reduction
Epoxidation
cyclopropanation

FIG. 38

Synthesis of Benzothiazole Epo490 and dEpoB.

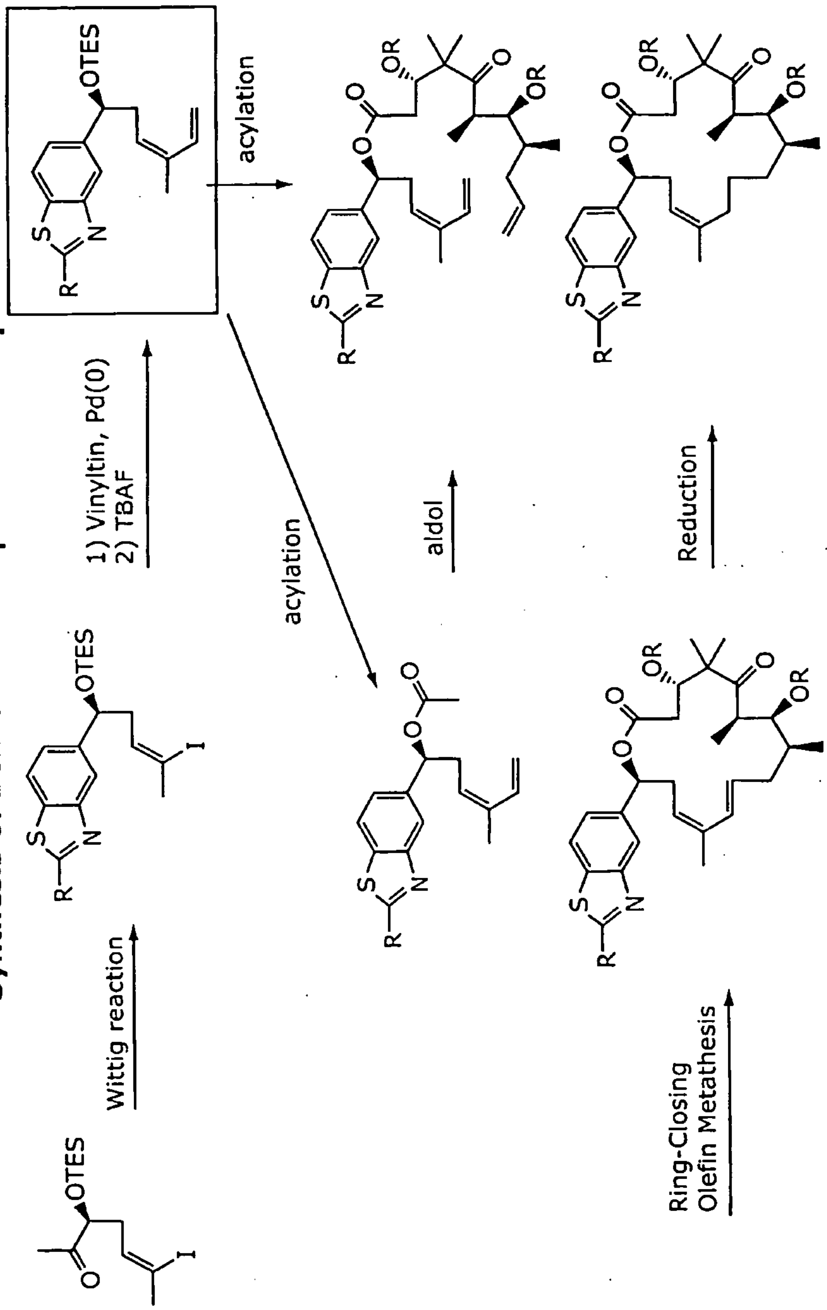


FIG. 39